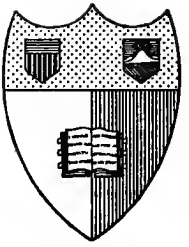
OXYHÆMOGLOBIN

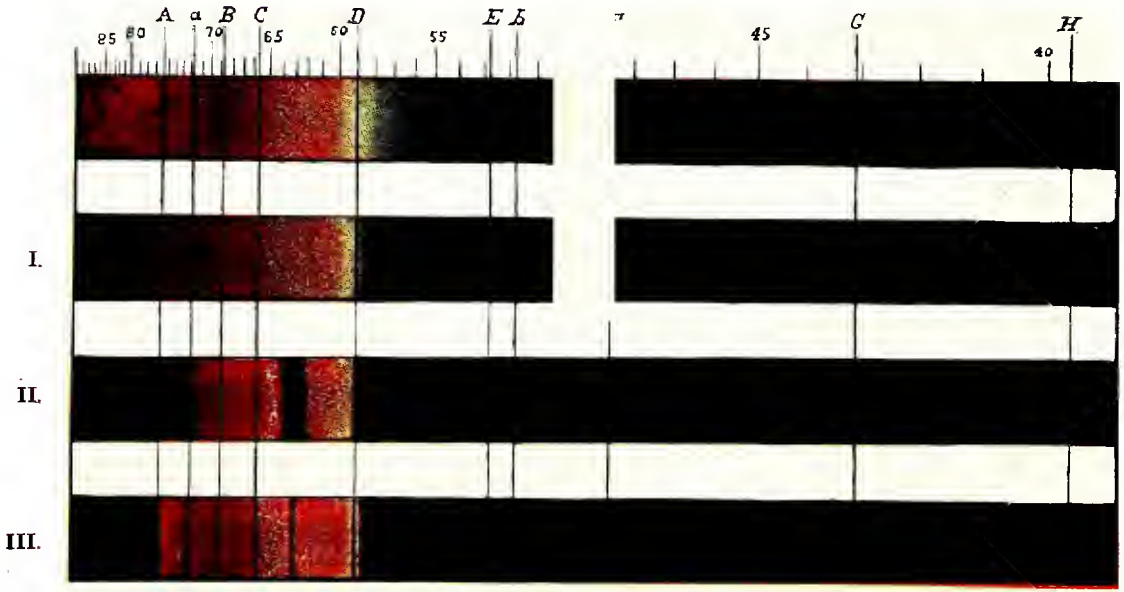
AND

ALLIED PRODUCTS.

FREDERICK STEARNS & CO.



ABSORPTION SPECTRA OF OXYHÆMOGl N, METHÆMOGLOBIN AND HÆMOFERRUM.



I.—Spectrum of Oxyhæmoglobin. II.—Spectrum of Methæmoglobin. III.—Spectrum of Hæmoferrum.

AN ILLUSTRATED MONOGRAPH

ON

OXYHÆMOGLOBIN

AND

ALLIED PRODUCTS.

PART I.—OXYHÆMOGLOBIN, ALBUMINATE OF IRON, AND PEPTONATE OF IRON. —STEWART.

PART II.—THE ABSORPTION OF IRON IN THE ANIMAL BODY. —MACALLUM.

PART III.—THE HÆMOGLOBINS AND RELATED PRODUCTS OF THE MARKET.—WILLIAMS.

PUBLISHED UNDER THE DIRECTION OF

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SCIENTIFIC DEPARTMENT.

FREDERICK STEARNS & CO.

F. E. STEWART, M. D., Ph. G., DIRECTOR.

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PHARMACEUTICAL ASSAYING—
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INTRODUCTION.

This monograph emanates from our Scientific Department and is in no sense of the term an advertisement.

Inasmuch as we have no exclusive control of the manufacture and sale of any article herein mentioned, and as the treatise is solely designed to advance the science of medicine and allied beneficent arts, we believe that the most enthusiastic "altrurian" can take no exception to it.

A word of explanation regarding our Scientific Department.

We organized it upon the theory that, pharmacy being a branch of medical science and practice, the standing of the manufacturing pharmacist, like that of the physician, should be guaged rather by his contributions to science, than by the extent of his business. All commercial considerations have, therefore, been eliminated from this department, which we maintain solely for the gratuitous dissemination of knowledge by the publication of the investigations of modern scientists. It is, however, far from being a mere Bureau of Publication, in that the outside investigator is supplied with material for original laboratory or bedside experiments, and is, in special cases, remunerated for his labors.

We make no false pretenses; we have a natural desire to augment the prestige of our house, and the conduct of this scientific work brings us into a frequent contact with leaders in medicine and pharmacy which necessarily encourages friendly relations and ultimately swells our list of patrons. Again—the discussion of the therapeutic value of drugs of which we, in common with other houses, place new preparations upon the market, increases the general demand and incidentally our own output.

Before a remedy is introduced to the profession at large, it should be thoroughly tested in laboratory and hospital practice. Private practitioners will not bring this about by merely ignoring preparations which have not undergone such tests. They should rather devote their energies to enforcing the proper performance of duty in this regard by physicians connected with laboratories and hospital dispensaries.

We hold that the manufacturing pharmacist is comparatively little to blame for the present situation in regard to proprietary medicines in that he has simply supplied what was called for, leaving it to the profession to create a demand for something better. This the latter can now do by co-operating in the work of our

Scientific Department. As an evidence of liberal spirit, we offer to remunerate them for such work as may be done under our control, or in the laboratories of the universities, or in large hospitals. The proper performance of the scientific work undertaken has been assured by the careful selection of a staff of experts in the theory and practice of medicine, pharmacy and chemistry.

The lofty standard set for the department will be persistently maintained. It will not be permitted to degenerate into a mere Advertising Bureau. Conducted as it is by high-grade physicians and pharmacists who can be held personally accountable for their work, we feel justified in soliciting the active participation of the profession in their labors.

TO PHYSICIANS.

The staff of the Scientific Department of FREDERICK STEARNS & Co. hereby extend a cordial and urgent invitation to practitioners who may desire information on any subject pertaining to therapeutics or pharmacy, to address their inquiries to the laboratory. If the problem submitted for solution is such as can only be passed upon by a specialist, we shall without delay place it before one of the numerous experts with whom we are in touch and the response will be promptly forthcoming.

PART I.

Oxyhæmoglobin.

BY F. E. STEWART, M. D., PH. G.,

Director of the Scientific Department of Frederick Stearns & Co. Formerly Lecturer on and Demonstrator of Materia Medica and Pharmacy, Jefferson Medical College, Medico-Chirurgical College, and Woman's Medical College, Philadelphia; Author of a Compend of Pharmacy; Member of the American Medical Association, American Pharmaceutical Association, Judicial Council Ninth International Medical Congress, etc.

Oxyhæmoglobin.

Hæmoglobin, the well known principle to which the blood owes its color, is chiefly of interest to the physiologist as an oxygen carrier, and to the therapist because of the iron it contains. Its administration in anæmia has been favored because of the avidity with which, when merely exposed to the air, it absorbs and combines loosely with oxygen, and second, because of the ease with which, when brought into relation with the oxygen free tissues of the body, it releases the oxygen for use in the respiratory processes. Unfortunately, this theory does not take into consideration the action of the gastric juice on hæmoglobin. Bunge says, "Hæmoglobin[1] splits up rapidly under the action of the digestive ferments; the iron separating as hæmatin. It is not known whether a part of the hæmatin becomes absorbed, as no quantitive experiments have been made to decide this point." Hæmoglobin would not act as an oxygen-carrier, if injected directly into the blood, for experiments have proven that[2] the hæmoglobin thus injected appears in the urine as urobilin, a product of the conversion of bilirubin. It is almost certain that bilirubin arises from the hæmoglobin.

The absorption of iron is being discussed by leading physiologists throughout the world. Bunge believes that no preparation of iron is absorbed, while Macallum has brought forward evidence which is almost overwhelmingly in favor of its absorption. The researches of the latter (published in the Journal of Physiology for April, 1894), are so interesting and valuable that we have included them in this Monograph. (Part II). Whether Iron is absorbed or not, this cannot affect its position as a therapeutic agent. Even Bunge himself, in his classical work of Physiological and Pathological Chemistry, page 94., after using arguments to prove that Iron is never absorbed, does not hesitate to say that "It is nevertheless noteworthy, that there are few remedies of the efficacy of which in cases of chlorosis, almost all physi-

[1] Text Book of Physiological and Pathological Chemistry, G. Bunge; translated from the second German edition by the late L. C. Wooldridge, M. D., D. Sc. London, Kegan-Paul Trench, Trubner & Co., 1890.
[2] Ibid. p. 375.

cians are so firmly convinced, as that of iron. It is not one of the remedies which are recommended one day, considered infallible the next, and forgotten in a short time thereafter, for the administration of Iron is as old as the history of medicine itself. Even those skeptics who doubt the value of all other drugs will assure us that chlorosis, which is frequently so obstinate, will almost invariably yield in a few weeks to a vigorous treatment with iron."

The next question is, how much iron should be administered? That, again, depends on whether it is absorbed or not. If it *is* absorbed, a very small amount would suffice to supply the blood with hæmoglobin. While the amount of iron in hæmoglobin is but 0.42 per cent, this is ample for therapeutic purposes if it be absorbed. If however, it is *not* absorbed, much larger doses of iron would have to be administered, in order to render the alkaline sulphides found in the intestine inert.

Another very interesting question bearing on the medicinal value of hæmoglobin is as to whether organic or inorganic preparations are the more readily absorbed. It has always been assumed that vegetive intervention was necessary to raise inorganic matter to the plane of animal life. It is because animals cannot subsist on inorganic material that they feed on vegetables or the flesh of other animals. Now, haemoglobin being an organic preparation, the probability of its absorption seems to be greater than is the case with inorganic preparations of iron. Being the form of iron found in the blood, it can be cogently argued that it must be peculiarly adapted for supplying the blood with iron. The comparative powers of absorption of inorganic and organic iron are discussed at length in Prof. Macallum's article.

Hæmoglobin and Oxyhæmoglobin.

Physiological text books of recent issue have discriminated more clearly between haemoglobin and oxyhaemoglobin. The method for preparing haemoglobin given in Foster's Physiology (3rd edition) furnishes oxyhæmoglobin rather than hæmoglobin. The crystals of haemoglobin have a dark red appearance, with a strong purple or bluish tint, unlike the brighter color of oxyhaemoglobin. The method referred to consists primarily of breaking up the corpuscles by the addition of chloroform or bile salts or by alternately freezing and thawing. The alkaline serum should be first removed, either by allowing the blood to clot or by centrifugally separating the corpuscles. The breaking up of the corpuscles results in an aqueous solution of hæmoglobin. The alkalinity of the solution can, if necessary, be reduced by the cautious addition

acetic acid, while the solvent power of the aqueous medium can be diminished by the addition of one-fourth its bulk of alcohol. When the blood of the dog, cat, horse, etc., is used, and the mixture set aside in a temperature of 0°C. in order to still further reduce the solubility of the haemoglobin, it readily crystallizes. When prepared from bullock's blood, however, crystals are very difficult to obtain. The solution of haemoglobin should, therefore, be concentrated in vacuo and dried on glass plates. The resultant product is pulverulent, or rather of a character resembling the scaly salts of the pharmacopoeia. If this process is carefully conducted, the finished product is almost pure oxyhæmoglobin. The only impurity present is a small amount of methæmoglobin, which in so far as it affects the medicinal properties of hæmoglobin, should hardly be called an impurity, for it contains as much iron as hæmoglobin itself.[1] Oxyhæmoglobin and methæmoglobin have exactly the same chemical composition, the only difference being that the oxygen is more loosely combined in oxyhæmoglobin than in methæmoglobin.

In other words, we may probably say, without being able to state exactly what has taken place, that, under certain conditions, the oxygen, loosely united to hæmoglobin as oxyhæmoglobin, becomes more stably combined and is not removable either by means of a vacuum, or of carbon-monoxide, or of a current of hydrogen; and, further, that the resulting substance (methaemoglobin) has the same composition and crystalline forms as oxyhæmoglobin, and may be reconverted into the latter body by suitable means, such as reduction by ammonium sulphide and subsequent oxidation.[2]

Composition of Hæmoglobin.

As Foster has pointed out, "[3]The exact nature of the proteid constituent of hæmoglobin has not as yet been clearly determined. It was supposed to be globulin (hence the name hæmetoglobulin, contracted into hæmoglobin), but though belonging to the globulin family, it has characteristics of its own; it is possibly a mixture of two or more distinct proteids. It has been provisionally named GLOBIN and is said to be free from ash." Hæmoglobin therefore must be classed as a food product which probably plays a similar part in nutrition to that of other proteid substances. Another interesting feature in relation to this product is that its alkaline solution is capable of being reduced by reducing agents; the spectrum changing at the same time. The reduced solution

[1] The Chemical Basis of the Animal Body; an appendix to Foster's Text Book of Physiology (sixth edition) by A. Sheridan Lea, M. A., D. Sc., F. R. S. p. 229.
[2] Ibid. p. 230.
[3] Ibid. p. 568.

will, like the hæmoglobin, take up oxygen again on being brought into contact with the air or oxygen. This would seem to indicate that the oxygen-holding power of hæmoglobin is connected exclusively with its hæmatin constituent.

"[1]By the action of strong sulphuric acid, hæmatin may be robbed of all its iron. It still retains the feature of possessing color, the solution of iron-free haematin being a dark rich brownish red; but is no longer capable of combining loosely with oxygen. This indicates that the iron is in some way associated with the peculiar respiratory functions of hæmoglobin; though it is obviously an error to suppose, as was once supposed, that the change from venous to arterial blood consists essentially in a change from a ferrous to a ferric salt.

As already stated, when administered as a medicine, hæmoglobin is split up by the gastric juice; its globin being converted into a soluble peptone (which is absorbed) while its hæmatin is set free. Hæmatin is a remarkably stable substance, strongly resembling iodine; or, if powdered, appearing dark or light brown according to the fineness of the powder. It may be heated to 180° without decomposition; but, by stronger heating, it is finally decomposed, liberating an odor of hydrocyanic acid and leaving a residue (12.5 p. c.) of pure oxide of iron. It is quite insoluble in water, alcohol, ether, chloroform or benzol; but is somewhat soluble in strong acetic acid, especially if warm. It is also soluble in alcohol, (not water) to which some acid has been added; and readily soluble in alkaline solutions, or in alcohol containing alkalies.[2]

Spectroscopic Assay.

As Oxyhæmoglobin prepared from bullock's blood does not crystallize readily, if at all, it appears on the market in the form of a more or less scaly powder, resulting from the method of drying it. The microscope, therefore, does not furnish a reliable method for determining its purity. This must be ascertained by means of the spectroscope, for a tolerably dilute solution is found to absorb certain rays of light in a peculiar and characteristic manner. The spectrum of oxyhæmoglobin may be seen by referring to the frontispiece of this monograph. Here it will be observed that "[3]a portion of the red end of the spectrum is absorbed, as is also a much larger portion of the blue end; but what is most striking is the presence of two strongly marked absorption bands, lying between the solar

[1] Ibid. p. 568.
[2] Ibid. p. 234.
[3] Text Book of Physiology by M. Foster, M.A., M.D., L.L.D., F.R.S. (Sixth edition). London, Macmillan & Co., and New York, 1891. Part II., p. 561.

lines D and E. Of these, the one towards the red side, sometimes spoken of as the band (*a*), is the thinnest, (and, in extremely dilute solutions, the only one visible) but the most intense. Its middle lies at some little distance to the blue side of D. Its position may be more exactly defined by expressing it in wave-lengths. As is well known, the rays of light which make up the spectrum differ in the length of their rays, diminishing from the red end where the rays are longest, to the blue end where they are shortest. Thus Frauenhofer's line D corresponds to rays having a wave-length of 589.4 millionths of a millimetre. Using the same unit, the centre of this absorption band (*a*) of hæmoglobin corresponds to the wave-length 578. The other, sometimes called *b*, much broader, lies a little to the red side of E, its blueward edge, even in moderately dilute solutions, coming close up to that line. Its centre corresponds to about wave-length 539. Each band is thickest in the middle, and gradually thins away at the edges. These two absorption bands are extremely characteristic of hæmoglobin. Even in very dilute solutions, both bands are visible; in fact they may be seen in a thickness of one cm. in a solution containing one grm. of hæmoglobin in ten litres of water. This is the case when scarcely any of the extreme red end, and very little of the blue end, is cut off. They then appear not only faint but narrow. As the strength of the solution is increased, the bands broaden, and become more intense; at the same time both the red end, and still more the blue end, of the whole spectrum, are encroached upon. This may go on until the two absorption bands become fused together into one broad band. The only rays of light which then pass through the hæmoglobin solution are those in the green between the blueward edge of the united bands and the general absorption which is now rapidly advancing from the blue end, and those in the red between the united bands and the general absorption at the red end. If the solution be still further increased in strength, the interval on the blue side of the united bands becomes absorbed also, so that the only rays which pass through are the red rays lying to the red side of *d*; these are the last to disappear, and hence the natural color of the solution as seen by transmitted light. Exactly the same appearances are seen when crystals of hæmoglobin are examined with a microspectroscope. They are also seen when arterial blood itself (diluted with saline solutions so that the corpuscles remain in as natural a condition as possible) is examined with the spectroscope, as well as when a drop of blood, which from the necessary exposure to air is always arterial, is examined with the microspectroscope. In fact, the spectrum of hæmoglobin is the spectrum of normal arterial blood.

When oxyhæmoglobin loses its oxygen and becomes reduced to hæmoglobin, either by submitting the solution to the air pump or adding reducing agents, such as a few drops of ammonium sulphide or of an alkaline solution of ferrous sulphate, kept from precipitation by the presence of tartaric acid, or even if, to the solution of oxyhæmoglobin, be added an unpurified solution of blood corpuscles, such as is afforded by the washings of a clot, for example, the oxygen in loose combination is immediately seized upon by the reducing agent. The two absorption bands now disappear, and, in their place, there is seen a single, much broader, but at the same time, much fainter band whose middle occupies a position about midway between the two absorption bands of the unreduced solution, though the redward edge of the band shades away rather farther towards the red than does the other edge towards the blue; its centre corresponds to about wave-length 555. At the same time the general absorption of the spectrum is different from that of the unreduced solution; less of the blue end is absorbed. Even when the solutions become tolerably concentrated, many of the bluish-green rays to the blue side of the single band still pass through. Hence the difference in color between hæmoglobin which retains the loosely combined oxygen, and hæmoglobin which has lost its oxygen and become reduced. In tolerably concentrated solutions, or tolerably thick layers, the former lets through the red and the orange-yellow rays; the latter the red and the bluish-green rays. Accordingly, the one appears scarlet, the other purple. In dilute solutions, or in a thin layer, the reduced hæmoglobin lets through so much of the green rays that they preponderate over the red, and the resulting impression is one of green. In the unreduced hæmoglobin or oxyhæmoglobin, the potent yellow which is blocked out in the reduced hæmoglobin, makes itself felt, so that a very thin layer of oxyhæmoglobin, as in a single corpuscle seen under the microscope, appears yellow rather than red.

Therapeutics.

Attracted by the remarkable results attained by the use of defibrinated blood quaffed at the butcher's shambles, I took up an investigation of this practice in 1879, and shortly [1]afterwards introduced *Sanguis Bovinus Exsiccatus* (dessicated blood). This was prepared in the following manner: Fresh bullock's blood was defibrinated in the ordinary way by stirring and then dried on iron plates in the form of scales. If done properly, an odorless product with an albuminous taste resulted. It dissolved readily in cold water

[1] A New Method of Rectal Alimentation by F. E. Stewart, M. D., Ph. G., Medical Record, Jan. 3, 1880.

forming a perfectly clear blood-red solution. After standing for some time, however, the scales lost their ready solubility and could be dissolved only with difficulty. Again, in the Medical Record, a few months later, I recommended defibrinated blood as a substitute for extract of beef. In the Therapeutic Gazette for January 1884, I again called attention to the use of this article for use as a therapeutic agent, under the caption "Hæmoglobin."

While I was conducting the experiments in my New York laboratory which led to the introduction of desiccated blood, Dr. Andrew H. Smith of that city was experimenting with defibrinated blood as a means of supplementary rectal alimentation at St. Luke's Hospital. When I called Dr. Smith's attention to the desiccated product, he immediately put it into use. During his experiments with it he sent me the following formula on a postal card:

℞. Sanguinis Bovini Exsiccati ..... ʒ viij
Aquæ ..... ℥ vj.
Spiritus Frumenti .....
Glycerine..... ....āā..... ℥ j
Misce. Sig. Tablespoonful three or four times a day.

This formula has been variously modified by the different manufacturing houses and we have in consequence several so-called "fluid" foods. These have had a very large sale in spite of their disagreeable nature. While open to many objections, they have certainly done good work in a great many cases.

In 1890, I attempted to improve on these fluid preparations by another modification of Dr. Smith's formula, using fresh defibrinated bullock's blood in place of the dried article. This I introduced under the name of hæmoglobin compound; the formula for the preparation containing three fluidounces each of defibrinated blood and extract of malt, and an ounce each of whiskey and glycerine. Since my introduction of this preparation, Frederick Stearns & Co. have brought forward an oxyhæmoglobin to which they have given the trade name "Hæmoferrum."

The dry preparations of hæmoglobin on the market certainly possess manifest advantages over those furnished in fluid form, although they lack the stimulating properties of the latter. This objection, is, however, easily overcome by administering small quantities of some stimulant.

The question as to the power of the various tissues of the body to absorb albuminous substances is one of great interest. Albumen, being a colloidal substance, will not dialyze through animal membranes outside the body, and it has, therefore, been urged that the action of the gastric juice is necessary to fit such substances for

absorption. The evidence against this theory, however, is strong. Eggs and other albuminous substances are successfully employed for rectal alimentation, and physiologists claim that the lower bowel contains no digestive ferment. Dr. Campbell, of Georgia, answered this objection by saying that a retro-staltic action was set up which carried the injected substance above the ileo-cœcal valve and that it was digested in the fluid of the small intestines. This suggestion, however, has not been generally accepted by the medical profession.

Some years ago, Dr. Andrew H. Smith, of New York, had under his care in St. Luke's Hospital, a man in the last stage of pulmonary phthsis, to whom an injection of 120 grammes of blood was given every evening. The patient died just after an enema had been administered. At the autopsy, the large intestine was found to be very evenly coated with thickened blood for a distance of nearly three feet. While this is an argument in favor of retograde peristalsis, there is no proof that the injected material was carried above the ileo-cœcal valve.

A more striking argument in favor of the power possessed by certain living tissues to absorb colloidal substances is furnished by the experiments of Ponfick, who published in "L'Union Medicale" in 1879 or '80 reports of his investigations in regard to intra-peritoneal injection of defribrinated blood. He reports five cases of profound anemia which were greatly improved by injection of from 15 to 20 ounces of defribrinated blood into the peritoneal cavity. While we are all aware that extravasated blood is absorbed in cases of surgical operations, it has also been claimed that the surface of the skin itself is capable of absorbing oils and fats applied by inunction. Cod liver oil is frequently used in this manner in the treatment of debility where the stomach is in an enfeebled condition. It is, however, doubtful whether the good effects should not be attributed to the massage accompanying the inunction.

Bunge has pointed out that [1] "We have been satisfied to account for the absorption of food from the alimentary canal by the laws of diffusion and osmosis, but we now know that, as regards osmosis, the wall of the intestine does not act as would a dead membrane. We know that the intestinal wall is covered with epithelium and that every epithelial cell is in itself an organism, a living being with the most complex functions. We know that it takes up food by the active contraction of protoplasm in the same way as observed in independent naked animal cells, such as amœbæ and rhizopods. Observations on the intestinal epithelium of cold-blooded animals

[1] Text Book of Physiological and Pathological Chemistry. p. 3.

have made it obvious that the cells grasp the particles of fat contained in the food by means of their protoplasmic processes; and that they incorporate the fat globules with the protoplasm of the cell, which finally passes them on to the commencement of the chyle-vessels. So long as this active intervention of cells was unknown, it was impossible to understand the remarkable fact that, although the minute drops of fat were able to pass through the intestinal wall, yet finely divided pigments, intentionally introduced into the intestine, remained quite unabsorbed. At the present time, we know that all unicellular organisms possess the power of selecting their food, of taking up the useful and rejecting the useless substances." How much these facts may account for the apparent power of absorption possessed by the mucous and serous membranes it is impossible to say.

Foster, in his classical work on [1]physiology, explains the structure and functions of the body in a comprehensive manner, using the simple organisms known to biologists as amœbæ as a type of the more complex organisms of the system. The amœbæ are almost wholly composed of undifferentiated protoplasm in the midst of which lies a nucleus (though this is sometimes absent). He calls attention to the fact that this protoplasm possesses certain fundamental vital properties. It is contractile, irritable and automatic, receptive and assimilative, metabolic and secretory; also respiratory and reproductive. The tissues of higher animals may be regarded as groups of peculiarly associated amœbæ. Their accompanying physiological phenomena depend upon the combination of the fundamental properties possessed by the original protoplasm. In the evolution of living beings, it has finally come to pass that, in higher animals and plants certain groups of the constituent amœbæd-iform units or cells have, in company with the change in their structure, been set apart for the manifestation of some of the fundamental properties of protoplasm, to the exclusion or at least to the complete subordination of the other properties. While one group of cells constituting the nervous tissue manifests the properties of irritability and automatism, with an almost complete absence of contractility and a great restriction of the other qualities, another group is characterized by the activity of its protoplasm in the secreting of digestive fluids, and in the promoting of the peculiar chemical and physiological changes known as digestion and assimilation. Is it not possible that other tissues in which these properties are only latent may become stimulated temporarily? Admitting

[1] Text Book of Physiology by M. Foster, A. M., M. D. etc., Phila. Henry C. Lee's Sons & Co. 1881.

this possibility, can we not account for the absorption and assimilation of food by mucous and serous membrane.

[1]Dr. Desiderio Varela, of Mexico, reports an interesting instance of the efficacy of this agent in anæmia. The patient was a young woman, 20 years of age, who had become excessively debilitated.

He says that every known preparation of iron and tonics had been tried in vain, and at last the situation became such that it was determined to adopt the procedure recommended in like cases by Drs. F. E. Stewart and A. H. Smith, of New York, viz.: Enemata of defribrinated blood. On the third day, after using four or five ounces per diem in this manner, a favorable change was noticed, and, about the fifth day, the stomach became tolerant of light foods, and a favorable prognosis was ventured upon. On the twelfth day all rectal alimentation ceased, and at the end of a month, she returned to her household duties.

Dr. A. Ernest Sansom contributed a valuable article to the Lancet (April, 1882), in which he recorded two very interesting cases which rapidly improved after the injection of defribrinated blood. One suffered with dilated stomach, intense anæmia and purpura, together with severe pain and vomiting; the other was one of probable renal calculi, though the diagnosis was less clear.

Dr. Thos. S. Butcher, in the Medical Bulletin for November, 1882, makes a strong plea in behalf of the administration of bullock's blood as a remedy in anæmia. He prefers to use the fresh blood (not defribrinated) as a drink immediately after the animal has been slaughtered, without waiting for it to become cool. He says that, while there is something repulsive to a delicate and fine lady in the idea of drinking the warm, fresh blood from a dying animal, yet these persons finally become accustomed to it, and do not object to the taste of the fluid. The treatment is resorted to three times per week; the amount first administered being about two fluidounces. This is gradually increased until a full glass is drank each time. The only addition allowed is a small quantity of salt, which makes it more palatable to some patients. Dr. Butcher says that, where the directions have been carefully followed out and the treatment persisted in for a sufficient length of time, the average gain in weight has been fully one pound per week, and the general results have been such as to warrant his urging upon the profession this most effectual line of treatment. He enumerates six cases of anæmia in which this method was employed; one patient gaining 36 pounds in 36 weeks, another 12 pounds in 12 weeks,

[1] Reprinted from the El Genio Medico-Chirurgico, Jan. 22, 1882, in La Escuela de Medicina, Mexico, March 1882, and in London Medical Record, April 15, 1882.

another 16 pounds in 16 weeks. Two others gained from 1¼ to 1½ pounds per week during 14 or 15 weeks, and another about 5 pounds a month. Some of these cases were far gone in the downward course of their disease, but in all health was perfectly restored.

As the result of five years experience at the Royal Hospital for Women and Children, Dr. E. O. Day reports favorably in regard to the use of bullock's blood in the treatment of delicate children. His method of preparing the blood is to prevent its coagulation by dilution with water; drying it by means of water bath and powdering in a mortar. The dose for a child is a teaspoonful during the day.

Dr. Guerder, of Paris, France, has been very successful with defribrinated blood in the treatment of various diseases. He gave the powder to 8 convalescents, all of whom had been affected with typhoid fever in a grave form. Three out of the eight were unable to retain it, but the other five convalesced much more rapidly than their companions. He reports equally good results in the treatment of convalescents from adynamic fever, anæmia and other debilitated conditions of the system. Of the 24 cases affected with chlorosis, in which he gave this remedy, two vomited it frequently and digested it with difficulty; two others, who were dyspeptic, suffered oppression of the stomach in consequence and disagreeable eructations which caused them to discontinue its use. In the remaining cases, the results were very satisfactory. One case of the number that he has reported will serve as an illustration of his successful application of this remedy.

"Case 3:—Mademoiselle Emilie M., æt. 17 years, in easy circumstances, is one of those chlorotic girls that are the despair of a physician. She has been treated by several physicians and by myself for two years past. Tonics and chalybeates of all sorts, as well as ferruginous mineral waters, have been successively administered without great results. Menstruation regular but scanty. For some time past the young girl, naturally tall and of stout build, was losing flesh very fast; complexion sallow; lips pale; could not bear fatigue. The least emotion, the slightest exertion, brought on violent palpitations of the heart. The appetite was quite gone, and the sight of food provoked nausea. If forced to eat, she was taken with vomiting immediately after; short dry cough, but nothing about the lungs to excite apprehension. I prescribed the powder of meat. The patient took it with some repugnance, and vomited it at once. After trying the meat powder unsuccessfully for two days (in spite of the willingness of the patient to submit to any treatment), I was compelled to give it up. I proposed "gavage" (artificial feeding), but she would not consent to it. I then had re-

course to the powder of blood. The treatment was commenced October 6th, at first in the dose of 3, then 6 teaspoonfuls a day. The first effect, singularly enough, after three or four days' treatment, was to cause the nausea to disappear, and the patient began to eat a little. At the end of a fortnight the gain was manifest, the appetite was good and the digestion normal, the palpitations were gone and she was able to walk out every day. The treatment has been continued ever since, with occasional interruptions, and the young girl is now quite well."

Dr. Guerder says that the therapeutic indications of this agent are extremely numerous. In a general way it may be said that it is always indicated when there exists impediment of the organism, whatever its cause and nature. In simple anæmia, a better reconstituent cannot be found. The perfect tolerance for this product manifested by delicate stomachs make it a precious resource. On account of its iron, and the ease with which it can be borne, it is an excellent remedy in chlorosis. It has been found of great value in the treatment of dyspepsia and diseases of the alimentary canal; it is a preventive and curative in the treatment of infantile diarrhœa; it has shown itself of value as a tonic and reconstituent in pulmonary phthisis, and, as Dr. Steibel says in his recent treatise, "In my experience the powder of defibrinated blood rapidly stimulates the appetite and the digestive functions. It advantageously replaces iron, especially in the sufficiently numerous cases in which the latter is not well borne. With this action as a chalybeate is combined another no less valuable. It is an excellent aliment, reduced to the smallest volume possible, and of perfect digestibility. * * * As a chalybeate, the blood powder presents the advantage of not provoking constipation. In all debilitated states of the organism it will prove a valuable therapeutic adjuvant and nutrient.

In my article on "Hæmoglobin as a Therapeutic Agent" (Therapeutic Gazette, 1884. p. 55), I fell into the same error that other writers on this subject have been guilty of. Referring to the most important functions of the red corpuscles, viz., that of carrying oxygen, I said: "But it is to the power of hæmoglobin to hold a large portion of oxygen in its embrace that this function of the red corpuscles is due, and hæmoglobin has this property to an equal extent when separated from the corpuscles. Its administration, therefore, places in the blood an agent capable of restoring its lost function of carrying oxygen. Respiration is again established, and the slumbering vital fires are again aroused into activity." The error was, however, quite natural under the circumstances. Before advancing the theory, I consulted with several leading physiologists

who supported my view. That is, we unanimously accepted the theory as being reasonable, but the article referred to contains the following admission: "I have not yet demonstrated my theory with scientific exactness." I was particularly impressed even at that time, by the fact that the effects of hæmoglobin administration were entirely out of proportion to what might be expected from the amount given; i. e. if its virtues were to be attributed to the value of blood as a nutrient alone. I had seen such remarkable results from the administration of desiccated blood in 20 grain doses, three times a day, that I began to suspect that it was sometimes absorbed without being acted upon by the gastric juice, and that its effects were due to its going directly into circulation.

The Medical Record (1884) says, "our ideas of providing nourishment for patients with weak and irritable digestive or ns have been somewhat revolutionized of late years by the remarkable results obtained by Drs. Débove and Dujardin-Beaumetz with their methods of forced feeding. They have clinically demonstrated that proper nourishment plays no unimportant part in the therapeutics of certain chronic and convalescent states." The editor calls attention to the fact that indications for forced feeding are presented frequently in practice; much more frequently indeed, than many will admit. He says that "the splendid results obtained by S. Weir Mitchell, and L. Playfair from systematic feeding, show that its application is not limited to that class. Many cases of hysteria. anæmia, chlorosis, and convalescence from acute diseases and organic affections are accompanied by loss of appetite and even disgust for food, due in great part to the fact that the stomach has become so unaccustomed to the presence of food that it has partially lost its digestive power, the best restorative of which is food in small quantities frequently repeated." The Record then refers to the various meat powders and to the excellent results obtained by Guerder in the use of dried blood, suggesting the positive probability that the iron in the latter constitutes an important factor in its usefulness; its proportion of 0.30 part per hundred being sufficiently large to represent additional doses of iron. Débove and Dujardin-Beaumetz had indifferent results from the use of dried blood. That Guerder attributes to the large quantities which they administered and their faulty methods of preparing it. Indeed, their results with his preparations were highly satisfactory. Guerder had administered it in 51 cases. Of this number 44 took it well and without inconvenience for several weeks. The convalescents from typhoid fever were unable to retain it at all, while to the remaining 4 chlorotics it was disagreeable, producing unpleasant sen-

sations in the stomach and was sometimes vomited unchanged after several hours. Guerder not only obtained excellent results in chlorosis, convalescence from acute diseases etc., but the effects upon phthisical patients were most remarkable. When the disease was not far advanced, there was invariably an early increase of strength, weight and appetite.

Oxyhæmoglobin as a Pharmaceutical Product.

If the favorable results attending the administration of bullock's blood can be properly attributed to oxyhæmoglobin we have, within reach, a method of securing these results without the unpleasant associations attending the use of the blood itself as a therapeutic agent. By way of experiment, oxyhæmoglobin, ("Hæmoferrum" brand) prepared in the manner already described, was administered in three grain doses, twice in 24 hours to 72 persons, in order to determine whether or not it would produce unpleasant effects. This test, which was prolonged for one week, demonstrated that such doses will not, in the large majority of cases, disturb the stomach or bowels. Furthermore, that, being neutral in reaction, it can be administered indefinitely without injuring the teeth; that, being non-astringent, it will not produce constipation, and that, unlike other preparations of iron, it will not cause headache. It is true that the week's experiment developed a few cases of slight gastric disturbance, headache, diarrhœa, and constipation; but these could not be fairly attributed to the hæmoglobin. It would be very remarkable to find 72 persons, among whom there were no cases of dyspepsia or constipation. Twenty-three persons of the 72 reported that it increased the appetite, and, of these, 18 slept better while taking the medicine. As these people were all in comparatively good health and eating three square meals a day, the effects were probably due to imagination. We all know that, if sugar and water were given to a hundred patients with the statement that they might expect certain effects, a large portion would be confident that they experienced these effects. On the other hand, what physician has not been exasperated by the powerful imagination of patients, who ascribe every unpleasant symptom to his medicines? As the general results from these preliminary tests were favorable, the preparation was sent to a number of physicians in Detroit for clinical test. Dr. Hal C. Wyman tested it in the Detroit Emergency Hospital and reported that in his hands the preparation proved a "splendid" tonic. Dr. E. B. Smith reported that he was pleased with the good results obtained in his private practice, and at the East Market Dispensary. Dr. Oscar Le Seure used it in a number of cases of anæmia with

satisfactory results. He reports "The preparation is well borne by the stomach and improvement is rapid." Dr Dayton Parker used it in the wards of Emergency Hospital with gratifying results, particularly so in a case of anæmia following ovariotomy. Dr. John Lee reported equally good results in the outdoor clinic of St. Mary's Hospital. Dr. P. M. Hickey reported the case of Miss D, aged 18, who was referred to him for treatment Oct. 31, 1893 in a chlorotic condition. Upon physical examination, an anæmic murmur was discovered. She was placed upon oxyhæmoglobin for some weeks, at the end of which time the anæmic murmur disappeared, and her condition was so improved that she reported herself well and resumed her work. Dr. Hamilton E. Smith states that he used the preparation with satisfactory results. Dr. W. R. Chittick, in his experiments at St. Mary's Hospital, found oxyhæmoglobin to be the best possible form in which to administer iron, and as valuable in point of assimilation as any other form he had ever tried. His experience demonstrated that it was acceptable to the most delicate stomach.

The preparation was subsequently sent to a number of physicians in different parts of the country in order to verify the observations made in Detroit. Dr. J. C. Martin, of Kansas City, Kas., found it of value in the treatment of nervous exhaustion. Dr. J. M. Shaffer, of Keokuk, Ia., employed it with satisfactory results in cases where the usual iron preparations offended the patient. Dr. P. G. Gober, of Alma, Wis., reports good results in anæmia and recommends the preparation on account of its easy assimilation by weak stomachs.

Among the many physicians who have tested the "Hæmoferrum" brand of oxyhæmoglobin with equally good results may be mentioned Drs. F. S. Stuart, Buffalo, N. Y., J. F. O'Keefe, Mt. Clemens, Mich., L. B. Weathers, San Antonio, Texas, Wm. A. Dixon, Alpena, Mich., D. W. Byers, Reading, Mass., J. W. Wiggenton, Beebe, Ark., W. L. Wilson, Midland, Mich., T. A. Pearson, Hopewell, N. J., W. F. Reed, Cheboygan, Mich., T. C. Giroux, Brooklyn, N. Y., Geo. W. Stewart, Saginaw, Mich., A. C. Tuchler, San Francisco, Cal., J. W. Hauxhurst, West Bay City, Mich., H. Schoenfield, Trenton, O., M. C. Kelly, Mt. Clemens, Mich., E. K. Thompson, McPherson, Kas., M. C. L. Kitchen, Saginaw, Mich., Frances Mammell, McPherson, Kas., B. H. Beckwith, Saginaw, Mich., Lewis Rochet, Fremont, O., C. H. Sample, Saginaw, Mich., E. S. Blair, Correctionville, Iowa, C. D. Cope, Ionia, Mich., J. O. Chisholm, New Concord, Ohio, and many others. The preparation has been tested by several hundred physicians with equally good results.

Occasionally untoward effects are reported; for instance, in a small proportion of cases, the oxyhæmoglobin in pill form did not agree with the stomach and could not be taken; others again did not find the rapid improvement from its use which has been claimed by a great majority.

The bulk of the evidence is, however, entirely in favor of oxyhæmoglobin, because, while easy of assimilation, it is free from the disagreeable effects usually attending the administration of inorganic iron compounds.

For instance, Dr. Chas. E. McCallum, of Midland, Mich., makes the following interesting report:

"Miss. L., Aged 17. Had never menstruated. Weight 85 pounds. Tall and slender. Complexion almost transparent, with a greenish cast of countenance. Had rheumatism of the knee joints. Both were considerably swollen, and although she did not suffer any very severe pain, the joints were so much stiffened as to make locomotion very difficult. Had considerable palpitation of the heart and vertigo, also insomnia. Pulse very small and weak.

"Sept. 1st, 1894, was placed on oxyhæmoglobin ("Hæmoferrum" brand). Dose—One pilloid three times daily before meals and one at bed-time.

"Sept. 21st. Had gained 5 pounds. Appetite improved. Slept better. Did not complain of knee joints so much. Swelling had almost disappeared in one. Color was slightly improved. Countenance brighter. Dose increased to six pilloids per day.

"Oct. 20th. Had gained 12 pounds and was an entirely different looking individual. She had also menstruated two days. Menstrual blood quite bright in color. Knee joints almost entirely well and she was taking outdoor exercise daily.'

Dr. Chas. W. Ellis, of Saginaw, Mich., reports as follows.

"E. P., female, sixteen years of age, has been suffering from chlorosis accompanied with irregular heart action, defective appetite and general feeling of lassitude and despondency. She was placed upon oxyhæmoglobin ("Hæmoferrum" brand) with a satisfactory result. This preparation has been fairly tested in this case and I can cheerfully and heartily bear testimony to its excellence as a remedy when other forms of iron cannot be taken."

Dr. W. J. O'Reilly, attending physician, St. Mary's Hospital, Saginaw, Mich., reports:

"In September 1894, I was called to see Miss. M. B., who had spent nearly the whole previous twelve months in bed under treatment by gynecologists for uterine displacement and ovaritis. The constant retention of a pessary had produced pelvic cellulitis and an abscess which had not been entirely cured.

"I found her in an anæmic weakened condition and suffering from gastric catarrh, obstinate constipation etc. She had been obliged to use a laxative daily for twelve months and had been kept on a milk diet for several months by direction of previous medical attendants.

"The condition of her stomach was such that ordinary preparations of iron bid fair to aggravate the gastric catarrh and constipation. I therefore prescribed oxyhæmoglobin ("Hæmoferrum" brand) and solid food. She at once showed a marked improvement which had continued up to date (November 1st). She now walks from her home to my office for consultation.

"A remarkable result attending this treatmeut is that, since taking oxyhæmoglobin, she has been able to dispense with laxatives or cathartics and her anæmia has entirely disappeared."

It seems to be conclusively proven that all the good results attending the employment of defibrinated blood are obtained when oxyhæmoglobin is administered in doses of from three to five grains, three or four times a day. Oxyhæmoglobin, therefore, may be considered as occupying a relation to defibrinated blood similar to that which quinine bears to cinchona. Oxyhæmoglobin may, pharmaceutically speaking, be termed the "active principle" of blood. The use of this "active principle" does away with the large amount of inert material represented by the stroma of the corpuscles, serum, and other constituents of blood, and also with the disagreeable associations connected with blood administration.

The best form for administering oxyhæmoglobin seems to be that of pills, coated with a soluble preparation of gelatin. I would like to caution the profession, however, not to demand more from oxyhæmoglobin as a therapeutic agent in anæmia than can reasonably be expected of iron. It is folly to look for a specific for anæmia in any one agent, and iron is only one of the remedies to be used in this pathological condition. The cause should be sought for and removed when possible. The patient ought to be supplied with nutritious food largely of meats. Outdoor air, hydro-therapy, change of occupation and scene, improved hygienic conditions, etc., etc , should be employed together with iron in the treatment of anæmia and other debilitated conditions of the system. The physician should not rely on iron alone, no matter how much the particular preparation thereof has to recommend it. The question is not, "Is oxyhæmoglobin a specific in anæmia?" but "Is oxyhæmoglobin a better form of iron than the inorganic and organic preparations usually employed?" The mass of evidence accumulated in the manner hereinbefore indicated will, we believe, justify an affirmative response to this query.

Albuminate of Iron.—Most compounds of iron possess a decided astringency and respond to the characteristic test with ferricyanide of potassium. The styptic property, together with the excessive acidity of the most popular of these iron preparations, renders their universal use impossible. Efforts have been made from time to time to produce an iron preparation which would have all the therapeutic properties of iron and still be free from its most objectional features.

Dieterich and Barthel[1] found that iron could be made to unite with such organic substances as lactose, mannit, inulin, dextrose,

[1] Pharm. Rundschau. 1888, p. 39.

gelatine, albumin, and peptone, and further that these compounds would not respond to the usual iron tests. This reason, together with the fact that they were not astringent, led these chemists to designate these preparations and others of a like nature as "indifferent" iron compounds. Of these, the albuminate and peptonate of iron have become very popular in America, as well as in Europe where they were originated.

The albuminates of different manufacturers have been found to contain valuable percentages of iron, and some of these were not even permanent preparations. Quite recently a new artificial albuminate of iron has appeared, which is claimed to contain a constant percentage of iron. While this is apparently true, this compound possesses the same property as the ordinary albuminates in that it is altered by the digestive juice into ferric and ferrous chloride. The most constant and definite albuminate of iron is doubtless the natural compound of the blood (hæmoglobin). Until recently it has not been possible to obtain it pure on a large scale. The action of gastric juice converts the iron into a compound called hæmatin, only a small portion of ferric chloride being formed.

Peptonate of Iron.—By gradually adding a solution of chemically neutral ferric chloride to a solution of beef peptone, the liquid will be seen to assume a dark red color, and finally a point will be reached when no response to the well known iron test with potassium ferricyanide can be obtained. Not until a mineral acid has been added can this test be obtained. Since, with a definite strength solution of a given sample of peptone, the combining proportions of iron and peptone are constant, we are justified in saying that a chemical compound and not a mere mixture has been produced. As evidence of this, we may mention that this soluble compound is entirely devoid of astringency, and does not corrode the teeth, as is the case with ferric chloride and most other iron compounds.

Peptone in the free state dialyzes readily through animal membranes, but when combined with iron does not. Free peptone responds to the well-known biuret reaction, but when combined with iron it does not; yet the least excess beyond that required to satisfy the affinity of iron immediately responds to the characteristic test. These facts establish beyond doubt that the products of proteolytic digestion unite chemically with iron.

Objection has quite recently been offered to the use of the term "Peptonate of Iron" since it does not represent a simple, definite, chemical substance. Strictly speaking, this expression would indicate a compound formed by the union of iron and peptonic acid, an acid which does not exist. Nevertheless the name "Peptonate

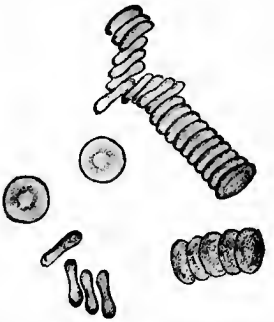
of Iron" is sanctioned by common usuage among scientific men and can therefore not be misleading. The absurdity of the terms "Peptonized Iron" and "Peptonated Iron" which have been suggested as more proper, becomes evident when they are associated with "Peptonized" or "Peptonated Milk." Peptonization is a process of digestion and no one would think of applying it to iron. Moreover, in using the expression "Peptonate of Iron," we are only following the example set by chemists the world over who invariably speak of the compounds produced by adding iron, and mercury to egg albumen, as albuminate of iron, and albuminate of murcury respectively. Instances are not wanting in the U. S. Pharmacopœia to sustain this view.

The composition of the peptonates, like that of the albuminates is probably not constant, for the reason that the so-called peptone of the market is a mixture of several products of proteid digestion. Popularly speaking the name "peptone" is applied to all the products of the action of pepsin and hydrochloric acid on albumin. But this digested proteid matter is a mixture of variable quantities of parapeptone, albumoses, hemipeptone, etc., of which only the true peptones are readily dialyzable. Probably each one of these products is capable of uniting with iron to form a definite compound. But peptone (so-called) being, as has already been stated, a variable mixture of all these proteid bodies, it can be readily seen why "Peptonate of Iron" is not an absolutely definite chemical compound. Doubtless if all conditions in the manufacture are equal, an apparently constant "Peptonate of Iron" can be made.

Whatever may be said of the absorption and assimilation of iron, "Peptonate of Iron" because of its peculiar properties, is quite generally admitted to be an exceptional form of iron ingestion. Being neutral, it does not derange the digestive organs and is therefore indicated where there is anæmia, chlorosis, or wherever prolonged use of iron is necessary.

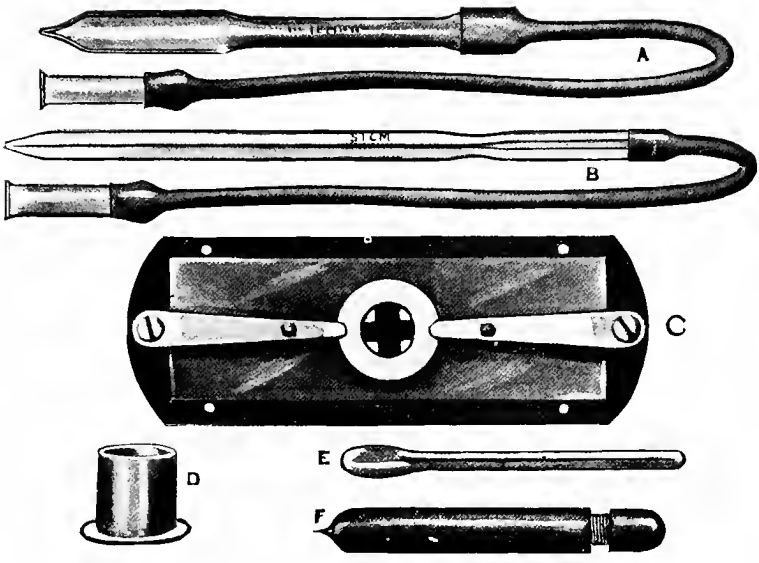
Method of Counting Red Blood Corpuscles.

The average number of red corpuscles in human blood may be probably put down at about 5 millions in a cubic millimetre (the range in different mammals is said to be from 3 to 18 millions), but the relation of corpuscles to plasma varies a good deal even in health, and very much in disease. Obviously the relation may be affected (1) by an increase or decrease of the plasma, (2) by an actual decrease or increase of red corpuscles. Now, tho former must frequently take place. The blood, as we have already urged, is always being acted upon by changes in the tissues and, indeed, is an index of those changes; hence the plasma must be continually changing, though



Human red blood corpuscles 1000 diameters.

always striving to return to the normal condition. Thus when a large quantity of water is discharged by the kidney, the skin, or the bowels, that water comes really from the blood, and the drain of water must tend to diminish the bulk of the plasma, and so to increase the *relative* number of red corpuscles, though the effect is probably soon remedied by the passage of water from the tissues into the blood. So again when a large quantity of water is drunk, this passes into the blood and tends temporarily to dilute the plasma (and so to diminish the relative number of red corpuscles), though this condition is in turn soon remedied by the passage of the superfluous fluid to the tissues and excretory organs. The greater or

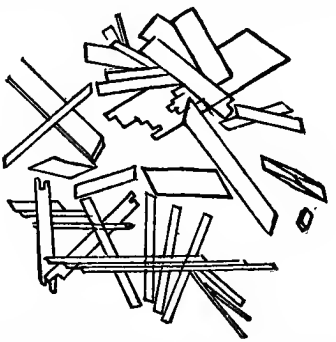


HÆMACYTOMETER OF GOWERS.

A, Pipette for measuring the diluting solution; *B*, capillary tube for measuring blood; *C*, divisions on slide, cover-glass and springs; *D*, vessel to mix solutions; *E*, mixer; *F*, pointed needle for sticking finger.

less number of red corpuscles, then, in a given bulk of blood may be simply due to more or less plasma, but we have reason to think that the actual number of the corpuscles in the blood does vary from time to time, This is especially seen in certain forms of disease which may be spoken of under the general term of anaemia (there being several kinds of anaemia), in which the number of red corpuscles is distinctly diminished.

The redness of blood is due to the presence of hæmoglobin. The degree of redness, may be influenced however, not only by the number of red corpuscles in each cubic millimeter of blood, but also by the amount of hæmoglobin in each corpuscle, and to a less degree by the size of the corpuscles. If we compare with a common standard, the redness of two specimens of blood unequally red, and then determine the relative number of corpuscles in each, we may find that the less red specimen has as many corpuscles as the redder



Crystals of Hæmoglobin from human blood.

one, or at least the deficiency in redness is greater than can be accounted for by the paucity of red corpuscles. Obviously in such a case the red corpuscles have too little hæmoglobin. In some cases of anaemia the deficiency of hæmoglobin in each corpuscle is more striking than the scantiness of red corpuscles.

The number of corpuscles in a specimen of blood is determined by mixing a small but carefully measured quantity of the blood with a large quantity of some indifferent fluid,—e. g. a 5 p. c. solution of sodium sulphate,—and then actually counting the corpuscles in a known minimal bulk of the mixture.

This perhaps may be most conveniently done by means of lassez. A glass slide in a metal frame, is ruled into minute rectangles, —e. g. $\frac{1}{4}$ mm. by $\frac{1}{5}$ mm.,—so as to give a convenient area of $\frac{1}{20}$ th of

of a square mm. Three small screws in the frame permit a coverslip to be brought to a fixed distance,—e.g. $\frac{1}{5}$ mm. from the surface of the slide. The blood having been diluted,—e.g. to 100 times its volume,—a small quantity of the diluted (and thoroughly mixed) blood, sufficient to occupy fully the space between the coverslip and the glass slide when the former is brought to its proper position, is placed on the slide, and the coverslip brought down. The volume of diluted blood now lying over each of the rectangles will be $\frac{1}{100}$th ($\frac{1}{20} \times \frac{1}{5}$) of a cubic mm.; and if, when the corpuscles have subsided, the number of corpuscles lying within a rectangle be counted, the result will give the number of corpuscles previously distributed through $\frac{1}{100}$th of a cubic mm. of the diluted blood. This multiplied by 100 will give the number of corpuscles in 1 cubic mm. of the diluted blood and again multiplied by 100 the number in 1 cubic mm. of the entire blood. It is advisable to count the number of corpuscles in several of the rectangles, and to take the average. For the convenience of counting, each rectangle is subdivided into a number of very small squares,--e.g. into 20,—each with a side of $\frac{1}{20}$ mm., and so an area of $\frac{1}{400}$th of a square mm.

PART II.

The Absorption of Iron in the Animal Body.

By A. B. MACALLUM, M. D., Ph. D.
Associate Professor of Physiology, University of Toronto.

Containing investigations as to the absorption of both inorganic and organic iron compounds.
(Illustrated).

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THE ABSORPTION OF IRON IN THE ANIMAL BODY.

By A. B. MACALLUM, M.D., Ph.D.,
Associate-Professor of Physiology, University of Toronto[1]. (Plate XI.)

The chemico-physiological relations of iron have been the subject of much speculation during the last fifty years. The chief difficulty in investigating the subject experimentally is the fact that, when iron enters into the composition of organic structures, it becomes "masked" and can no longer be detected by its ordinary chemical reactions.

The question is one of great practical interest, and Bunge's researches have in recent years done much to unsettle the medical dogma that the iron contained in drugs enters directly into combination with the red corpuscles to form hæmoglobin,

In 1890, an investigation on the formation of blood corpuscles in larval amphibia led me to conclude that hæmoglobin is formed from nuclein (the chromatin of histologists), and the iron-holding character of other nucleins has been demonstrated by Bunge[2], and Zaleski[3]. The discovery of micro-chemical methods[4] for detecting iron in cells has aided me in establishing the generalization that the most important of all elements in the life of every cell is an iron-holding compound. The prozymogens, if not the zymogens themselves, also contain iron.

I regard Bunge's theory of the direct conversion of iron-containing nucleins in the food (hæmatogen) into hæmoglobin as extremely doubtful, but the whole question of the synthesis of organic iron compounds is in an uncertain state, chiefly owing to our ignorance of the constitution of the nuclein molecule. There are, however, certain allied questions which can be more readily answered, and I have attempted in the following pages to determine first whether or not inorganic compounds of iron are absorbed, and secondly whether certain organic compounds of iron are absorbed.

[1] The expense attending the investigation of the absorption of organic compounds of iron (chromatins) was generously defrayed by a grant from the Elizabeth Thompson Science Fund.

[2] *Zeit. f. Physiol. Chem.* Vol. IX., p. 49, 1885.

[3] *Ibid.* Vol. X., p. 453, 1886.

[4] Macallum. *Proc. Roy. Soc.* Vol. L., 1891.

The second part of my paper must however be regarded as a preliminary communication, as my work in this direction is still incomplete.

I. ON THE ABSORPTION OF INORGANIC IRON COMPOUNDS.

The literature of this subject is very abundant but it consists largely of clinical records. The only papers, however, which I will mention are those which embrace observations on the quantitative estimation of iron ingested and excreted. In these the difference between the amounts is relied on to show whether iron salts are absorbed or not. The principal researches of this nature are those of Kletzinsky[1], Hamburger[2], Gottlieb[3], Kunkel[4], Kumberg[5], Busch[6], Marfori[7], Coppola[8], and Stender[9].

A careful perusal of these papers will convince the enquirer that the methods adopted are not calculated to answer the question, and that the various observers obtained different results.

In none of the series of experiments referred to, however, was the micro-chemical method employed to any extent except in those of Stender, although Kunkel's experiments on the livers of mice fed with iron indicate how valuable such a method would be in results, and it seemed to me that it would be easy to determine with it whether there is absorption of iron salts, and if so, in what manner and through what physiological agents it takes place.

Influenced by these views as to the value of the micro-chemical method, I made a number of experiments with guinea-pigs, kittens, lake-lizards and *Amblystomata*, using different preparations of iron and administering them either with or without food in various doses. At different intervals, after administering a preparation, or during the course of feeding with the preparation, the animal was killed, the abdomen opened, parts of the intestine, liver, spleen and kidney were removed, put directly into 95 per cent. alcohol or, after hardening for ten minutes in a saturated solution of corrosive sublimate, into alcohols successively of 50, 70, and 95 per cent. strengths. For control purposes teased-out portions of the fresh mucous membrane of the intestine were treated with ammonium sulphide and examined under the microscope. After the hardening was completed in alcohol, sections were made of the various parts

1 *Zeitsch. d. Gesellsch. der Aerzte zu Wien*, 1854, ii. 281.
2 *Zeitsch. f. Physiol. Chem.* Vol. II. 1878, p. 191.
3 *Arch. f. Exp. Path. u. Pharm.* Vol. XXVI. p. 139, 1889; *Zeitsch. f. physiol. Chem.* Vol. XV. p. 371, 1891.
4 *Arch. f. d. ges. Physiol.* Vol. L. p. 1, 1891.
5 and 6 Kobert. *Arbeiten des Pharm. Inst. zu Dorpat.* Vol. VII. 1891.
7 *Arch. f. exp. Pathol. u. Pharm.* Vol. XXIX. p. 212, 1891.
8 *Rendiconti della R. Acad. dei Lincei.* Vol. VI. p. 362, 1890.
9 Kobert. *Loc. cit.*

either by the free hand (liver and kidney), or by the paraffin or celloidin methods. Sections made by the paraffin method from tissues hardened in corrosive sublimate were fixed on the cover slip by Gaule's method, and the paraffin having been removed by the usual process, they were immersed in a mixture of equal parts of solutions of hydrochloric acid and potassic ferrocyanide of 0.5 and 1.5 per cent. strengths respectively. Here they were allowed to lie for about ten minutes, then they were washed in distilled water, dehydrated, cleared in cedar oil and mounted in benzole balsam. Sections made with the free hand, or with the celloidin method, were either mounted on the slide in a mixture of glycerine and ammonium sulphide and examined under the microscope, or placed in a ferrocyanide mixture for ten minutes, then carefully washed in distilled water, dehydrated in alcohol and, after clearing in oil of cedar, mounted in benzole balsam. Portions of the intestine hardened in alcohol were also put in a mixture of alcohol and ammonium sulphide in the proportion of 5 to 1. Material hardened in corrosive sublimate, on account of the presence of this reagent in the tissues, gave no useful preparations with ammonium sulphide, but they furnished, nevertheless, instructive ones when treated with the ferrocyanide mixture. In studying the preparations made with ammonium sulphide, I teased out portions of the mucosa and mounted them in a mixture of glycerine and ammonium sulphide. The glycerine retards the evaporation of the sulphide, and such preparations may be kept weeks and even months. In the case of the liver, spleen and kidney teased-out preparations yielded nothing of value, but sections of alcohol material, made sufficiently thin with a little practice by the free hand, were useful when mounted in the glycerine sulphide mixture. I have found that material hardened in alcohol furnished the best preparations, and that it gave results not equalled by those given by material fixed with corrosive sublimate, or those obtained by the use of ammonium sulphide on fresh material. The latter method is apt to lead to error, since the living or non-hardened tissues are slowly penetrated by ammonium sulphide, and when the penetration does take place, the cellular elements are more or less altered, giving confusing results. Alcohol has the advantage that it hardens rapidly and does not extract the salts of iron which are in the tissues in an albuminate form. In order to prevent diffusion of iron salts from without into the mucosa of the intestine, the latter, laid open immediately after removal, was in every case quickly washed free from adherent food matters and then dropped into alcohol.

In guinea-pigs there is absorption of iron in the intestinal

mucosa. This is readily seen in well-fed animals, less readily so in those whose stomachs and intestines are almost empty. The intestinal mucosa, after treatment with alcohol and when tested with ammonium sulphide, acquires a more or less dark color, due to the formation of sulphide of iron, which, under the microscope, is seen to be limited to the sub-epithelial portions of the tips of the villi. On closer examination the iron is found to be deposited in leucocytes which, in their disposition, form together a cap, as it were, for the extreme end of the lacteal vessel. The dark green reaction is not uniformly diffused through each cell, the nucleus being free from it, while, in addition to that present in the cytoplasm as a whole, there are masses in it which yield a greater intensity of color. The leucocytes are not as numerous immediately under the membrane on which the epithelium rests. They may occasionally be found between the epithelial cells of the tips of the villi; not, however, as much loaded with iron as those are which are found about the end of the lacteal. The iron of these cells originates from the food in great part, for, if the animal be kept without food for a week, the tips of the villi give but a feeble reaction. What is present in such preparations is derived from the bile, and this was shown by the results obtained from feeding guinea-pigs with egg-yolk. The latter, according to Bunge, contains but a trace of inorganic iron—and when it is fed to the animal but a feeble reaction for inorganic iron is obtainable in the villi. When, however, a trace of ether is added to the yolk given, the amount of bile poured into the intestine becomes greater, the absorption more vigorous, and then one finds the tips of the villa give a marked iron reaction almost as distinct as that present in the animal fed on its ordinary diet. This iron must therefore be derived from the bile. In all well-fed animals the iron reaction obtained is the more marked the nearer the part examined is to the pyloric opening, and at a distance of from seven to ten inches from it, it is usually absent altogether.

In ordinary guinea-pigs it is indeed very seldom that one finds inorganic or albuminate iron in the epithelial cells themselves. In order to meet the objection that possibly the iron in the lymph cells is that in the process of excretion, and also to determine how the iron compounds reach these elements, I fed several animals, which had fasted for about four days, with various preparations of iron, but using for this purpose specially some of the commercial "peptonate" of iron of which about 100—200 mgrm. were administered *per os* daily to each animal. The intestinal mucosa of animals so fed for three days became black in the ammonium sulphide solution, and on examination with the microscope the reaction, as before,

was almost confined to the tips of the villi, which were similarly affected down to the distal end of the small intestine. It was further found that the epithelial cells themselves covering the tips were loaded with iron; the leucocytes were massed below in great numbers and a large number had wandered between the epithelial cells in such a way as, in many of the villi, to displace and distort the cells. In very thin sections treated with the ferrocyanide mixture and mounted in balsam, the distribution of the iron was more clearly seen. Sometimes, in the epithelial cells, the blue reaction was a diffuse one with blue granules collected in groups here and there in the cell. In some instances it was found in the inner end of the cell chiefly, while again the protoplasmic processes in the hyaline border gave an intense reaction. Fig. 4 shows some of these details distinctly. In this are represented three cells, in two of which the inner ends appear loaded with iron and they were fixed in the act of transferring it to the underlying tissue. This involves an internal secretion, a process that plays an extensive part in absorption. The iron compound appears to be secreted in a soluble form, for I found that the underlying elements, the connective tissue fibres, yielded frequently a deep homogeneous blue reaction when the lower ends of the epithelial cells gave the same. When the amount of iron in the epithelial cell is small, that part of it in the lower end is dissolved in the protoplasm, but when the amount is large, it appears to be precipitated in a granular form. The dissolved form of the iron compound is possibly an albuminate, but the character of the granular form is difficult to determine, and it is equally difficult to do so in the matter of the deposit in the form of granular masses in the leucocytes below, although on the ground that some of these cells win their way with their iron back into the blood vessels, it is allowable to believe that the iron-holding masses in them, in order not to impede their movements, must be of albuminate composition and not inorganic with fixed shape.

The further course of the absorbed iron it is difficult to demonstrate, unless iron salts are given for some time. It is easy, of course, to determine that the leucocytes take up some of it. That fact is the one the most prominently seen even when the iron is given in large doses and for a long time, but it is only in the latter case that one is able by micro-chemical means to show that another and probably more important method of transfer exists. When the excised serosa of the upper part of the small intestine from a guinea-pig, fed for some time with either the phosphate, chloride or "peptonate," was treated, after hardening in alcohol, with the acid ferrocyanide mixture and after the usual course of treatment

mounted in balsam, the venules appeared blue while the arterioles were unaffected. In all these cases the blue color was found in both the contents and the intima of the vessels, and where the venule was empty, in the intima alone. In the contents there was a very light blue in the red corpuscles, a white corpuscle here and there contained iron and the plasma was shown to carry it by a color deeper than that given by the red discs. When the dose was very great indeed the iron in the plasma took a granular form, at least in alcohol preparations, but in this condition the muscular as well as inner coat of the vessel was blue. These venules are radicles of the portal vein. In the liver inorganic iron was found in all the tissues of the peripheral zone of the lobules, in part in a granular form in the peripheral hepatic cells, but diffused mainly through both cell and nucleus of each element. When the dose of iron given was not great, then the iron was mainly if not wholly confined to the peripheral zone. With large doses a greater portion of the lobule was impregnated with iron, and other elements came prominently to view, especially in the "peptonate" preparations. There were leucocytes in the angles of the capillaries in all parts of the lobule but very frequently in the central portion, and their occurrence was manifested under the low power by the strong reaction which they gave for iron (fig. 5). Sometimes each cell was a mass of blue material, or it contained large blue masses. In others again, the cytoplasm had a diffuse blue tint with one or more clumps of iron-holding substance. Did these leucocytes come from the villi of the intestine? Some of them undoubtedly must have done so, for, as already stated, in the contents of the venules in the serosa of the upper portion of the small intestine there were a few iron-holding leucocytes. It is probable, however, from a difference in the arrangement and deposition of the iron in them, that a large number have taken up the iron from the plasma after they became entangled in the capillaries. Some of them appear to be so large as to occlude the capillary channels. What they do with the iron which they contain is a matter of inference only. They probably transfer it to the liver cells through the capillary wall, or, if they again become free, they pass with it on to the general circulation. They are apparently not much discommoded by the amount of iron which they contain, for I found similar iron-holding leucocytes of all shapes and sizes both in the spleen pulp and in the venules leading off from it.

The iron-holding leucocytes of the villi, when they leave the latter, probably pass into the transverse branches of the capillary network or into the collecting venule of the villus. Outside of the

villi the occurrence of iron-holding leucocytes, except in vessels, is extremely rare, although when excessive doses, as, for example, 0·5 grm. daily of ferric phosphate, were given, they were increased in number, owing to the saturation of all the tissues of the intestinal wall with iron.

Whatever iron salt was administered, whether the "peptonate," phosphate, chloride or sulphate, when the dose was small, i.e. under 50 mgrms., the evidence of its absorption was very plain in the villi of the upper end of the small intestine. When tho dose was larger, as in the case of the phosphate and "peptonate," its presence was observed in the villi far down in the intestine, but the reaction was the less distinct the more remote the villus examined was from the pylorus. When either of the two was given in very large doses, (0·5 grm. daily of the phosphate for example), the villi near the cœcum gave an intense reaction.

These results can be explained readily. The iron salt of the chyme, when the latter is thoroughly mixed with the biliary and pancreatic fluids, becomes wholly precipitated if the alkalinity of the two latter fluids is sufficiently great. The alkali present may be completely destroyed by a large quantity of iron salt in solution, and when this occurs the excess of the iron salt not precipitated and remaining in solution is absorbed. When the quantity in excess of that necessary to destroy the alkalinity is very great, all the villi of the intestine are in a position to absorb some of it. If on the other hand the dose is small, absorption in the upper end of the intestine is favored by the circumstance that the three fluids, chyme, bile, and pancreatic juice, do not immediately and intimately mingle and, therefore, the iron is not at once precipitated, some of it being absorbed before that occurs. The quantity of acid in the chyme is a factor of some importance, and when the iron given is in the salt form and not the oxide, the acidity of the chyme is not decreased, the acid of the salt displaced taking the place of the hydrochloric acid. When the oxide or the reduced metal is administered, their solution takes up a portion or all of the acid without contributing in turn to the acidity of the chyme, and, therefore, in the intestine the alkalinity of the bile and pancreatic juices goes farther in the precipitation of the salts of iron in solution in the out-poured chyme. The larger the amount of free acid in the latter, the greater must be the quantity of iron absorbed.

I have made some experiments also upon the absorption of iron in kittens. One of two from the same litter was given, through a pipette by the mouth, 120 mgrms., in solution, of tartrate of iron and ammonia and both were killed four hours after with chloro-

form. The villi of the second kitten gave absolutely no reaction for inorganic iron while in the other fed with the iron salt, the sub-epithelial portion of the tips of the villi gave a marked one. In both the amount of milk in the stomach was the same, the gastric contents in one still holding apparently the greater part of the iron administered.

It was noted in this and in other cases where the dose administered was comparatively small, how free the epithelial cells themselves were from inorganic iron. Although in the sub-epithelial portions of the villi in the kitten the amount of iron was large, the epithelial cells when fixed did not contain the slightest trace of it. The possible explanation is that the cells transfer with great rapidity the iron which they absorb, and that it is only when the cells are fatigued by overwork in this transference, that some of the iron absorbed is seen in them.

I have already referred to the collection of the leucocytes in the tips of the villi and to the invasion of the epithelial layer by leucocytes in the "peptone" preparations. This is shown in Fig. 2, representing an optical section of a villus treated with ammonium sulphide and glycerine. The migration into the epithelial layer does not occur in every villus, while in some exhibiting this appearance it may be more marked than in others. The epithelial cells were very often greatly affected by the invasion, for not only were they considerably displaced, but they were in such a condition of disintegration that in some stained sections the extreme tips of some of the villi appeared denuded of epithelium. Whether the intra-epithelial leucocytes were the cause of this disintegration or not, they contained in addition to iron a great part of the disintegrated material. An invasion of the epithelial layer by leucocytes was also obtained in the villi when a quantity of albuminate of iron, made according to Marfori's[1] method, was given to a guinea-pig. The animal had been fasting for three days when the powdered albuminate, suspended in water, was given and five hours afterwards it was killed. As this compound is insoluble in weak acids, but readily soluble in weak alkaline solutions, it was not surprising that the villi from the opening of the pancreatic duct to the cœcum gave clear evidence of its absorption, while the villi for half an inch beyond the pyloric valve contained no traces of it. The villi near the distal end of the small intestine contained as much iron, judging by the reaction, as those which were situated near the opening of the pancreatic duct. The number of iron-holding leucocytes was not great, yet they carried a full complement of iron, and in many

[1] *Arch. für exper. Path. und Pharm.* Vol. XXIX., p. 212, 1891.

of the villi a majority of them were intra-epithelial. Their presence in the latter situation was not, as in the "peptone" preparations, accompanied by a disintegration of epithelial cells, although the latter were frequently much displaced.

These results seem to indicate that combinations of iron and proteid influence in some way the activity of the leucocytes, and, in order to obtain further evidence on this point, observations were made on the lake-lizards (*Nectus lateralis*) in the laboratory. The animals used had been for over thirty months without food, and the intestinal cavities of two examined contained nothing more than small masses of inspissated mucus impregnated with bile, but the mucosa of the same gave preparations of which that represented in Fig. 3 is typical. There was iron present in small quantities in the outer ends of the epithelial cells, but it was in the leucocytes that it was most abundant, the cytoplasm of these containing, in addition to what was diffused through it, large granular masses impregnated with iron. Leucocytes giving such a reaction were found scattered all through the mucosa. In the liver and spleen iron-holding leucocytes were found, in the former in the angles of the capillaries and in the latter in the adenoid tissue. To determine if in these animals the leucocytes are affected as they are in guinea-pigs by the albuminate of iron, a quantity of the dried albuminate of iron was dissolved in a very weak solution of bicarbonate of soda and injected through the vent into the intestine of one. Eight hours afterward the animal was killed, the liver, spleen and intestine removed and put into alcohol. The latter organ was only partially opened. In the liver and spleen the amount of iron in the leucocytes was greatly increased and the liver cells gave a deeper diffuse reaction. In the sections from the intestine the leucocytes were found loaded with an excess of iron, so much so that the ammonium sulphide gave them the appearance of huge collections of greenish black granules. In some parts of the intestine they were collected in large numbers under the epithelium with a few situated in the latter layer, but in other parts, especially near the vent, where naturally the bulk of the injected fluid collected, they were present in very large numbers in the epithelial layer, many of them fixed in all shapes and while migrating with their load of iron compound into the cavity. The best preparations which I obtained, however, were those from the unopened portions of the intestine, for here the contents were retained in the sections. A very large number of leucocytes excessively charged with iron were found in the cavity and others were fixed in the act of passing into it, while in the sub-epithelial leucocytes the iron was abundant. In all the preparations

the epithelial cells themselves were comparatively free from the iron compound.

In guinea-pigs fed with the chloride, phosphate, or sulphate of iron, I have not found that the leucocytes were similarly affected, even when these preparations were given without food, nor does the iron present in the ordinary food of the animals produce a like effect, although in all these cases the leucocytes gave unmistakable evidence of the absorption of these compounds.

It follows from the results of those experiments that inorganic and albuminate compounds of iron are absorbed, at least in the portions of the intestine near the pylorus, and in all parts of the small intestine when the iron compound is not precipitable on mixture with the bile and the pancreatic juice. Undoubtedly also, sulphides in the bowel must remove from solution a quantity of iron proportionate to their abundance. In ordinary diet the extent of the mucosa which absorbs iron must be, in proportion to that which does not, remarkably small. In may possibly be that in the human subject when the iron is specially increased the extent of the absorbing surface is increased, the more so when there is a diminution in the amount of pancreatic and biliary fluids, a condition possible in anæmias. The view held by some that iron salts are not absorbed and that these exercise their effect by stimulating the mucosa to greater physiological activity, is opposed by the results of these experiments. If iron salts stimulate, it is because they penetrate the epithelium of the mucosa and in doing so are transferred to the underlying elements. In other words, they are absorbed. But the extent of the absorbing area is limited; so likewise must be the extent of the area supposedly stimulated and, therefore, the beneficial effects of the stimulation of the mucosa alone must be small. What then is the purpose served by the absorption of iron salts? Leaving out of consideration the possible answer that the iron of combinations becomes assimilated, that is, is united in the animal cell with other constituents to form what the histologist calls chromatin, we may discuss some explanations of its effects. The experiments with the feeding of "peptonate" and of albuminate of iron are, of course, too few to enable one to infer anything concerning the action of iron-holding proteids of this character but all the experiments without exception indicate that the leucocytes have a special affinity for inorganic and albuminate compounds of iron, and it is not too much to infer that this affinity involves a stimulating or chemiotactic effect upon the leucocytes, and that iron salts exercise an effect on other cells corresponding to their function.

The number of leucocytes which are engaged at any one time

in the absorption of iron is comparatively small, owing to the small extent of the intestinal mucosa bathed by a solution of an iron salt, and, therefore, the stimulating effect would appear to he small, but it must be remembered that, with the constant stream of iron-holding plasma and iron-holding leucocytes from the villi in the upper portion of the small intestine to the liver, spleen, etc., the other cells of the body, including leucocytes with little or no inorganic iron, are put in position to obtain some of that absorbed. There is also another way in which the question may be viewed. Inorganic compounds of iron, like those of calcium, potassium, and sodium, have been, since the dawn of animal life on the globe, constituents of its media, and of its food, and it is possible that the animal cell has, in acquiring a tolerance for them, accommodated its functions to their presence and has established with them a physiological equilibrium which it may be possible to maintain in the absence of such compounds. The view that the iron of inorganic combinations goes directly into combination with nuclein and albumin to form chromatin, is one that may in the future be proved correct, but whether it will happen so or not, it is not now, nor apparently will it be then, incompatible with the explanations of other possible functions of iron salts like those just referred to.

In investigating the absorption of iron I had opportunities for determining the mode of excretion, when the iron absorbed is in excess of the needs of the organism. The material illustrating this was obtained from two guinea-pigs, one of which was given a very large quantity of the "peptonate" preparation of iron, while the other received a correspondingly large dose of ferrous sulphate. The secretion of the Lieberkühnian glands of the animal fed with the "peptonate" gave an intense iron reaction, and this was the case not only with the small intestine but also with the cœcum and upper portion of the colon. In the superficial epithelium of the two latter parts there was no evidence of either absorption or secretion of iron compounds. In the cells of the Lieberkühnian glands no iron reaction could be obtained, nor was it obtained in the same structures in the preparations from the animal fed with ferrous sulphate, although in the latter case the secreted material in the lumina of the glands appeared to consist, in large part, of an iron compound. Possibly the explanation for this is that the cells rapidly transfer with the secretion the iron compound to the lumen and in excessively small quantities at any one moment. The kidneys in both animals gave a very slight diffuse reaction for iron not confined to any part of the organs. It was only in the animal fed with ferrous sulphate that the liver yielded more than ordinary evidence of the

excretion of iron. The periphery of the lobules gave an intense iron reaction partly diffused through the hepatic cells, and partly localized in granules situated in that part of each cell bordering on the bile capillary. The latter gave a feeble reaction for iron, especially at the periphery of the lobules. The epithelium of some of the bile duclets contained free iron, probably absorbed from the bile.

In man the liver and kidneys are the most active organs in the excretion of iron. This has been shown by the observations of Hunter[1], Mott[2], and others in cases of pernicious anæmia. In the liver and kidney from a patient who had died from a complication of troubles in which pernicious anæmia was supposed by some to be a factor, I found this excretion illustrated to a remarkable degree. The bile capillaries were, in some parts of the sections, filled and distended with secreted iron compounds. Where this was the case the cells surrounding a capillary were almost free from inorganic iron compounds, but where the capillary was feebly or not at all injected, then the portion of each cell touching the capillary was loaded with granules of a ferric compound. The sections of the cortical portions of the kidneys gave an exceedingly intense reaction for iron, which, on examination under the microscope, was found chiefly in the convoluted tubules, the cells of these exhibiting both a diffused and a localized reaction, the latter given by an abundant collection of granules distributed in that half of the cell adjacent to the lumen. The intestine in this case yielded no evidence whatever of the excretion of iron.

The results of experiments on guinea-pigs and of observations on cases of pernicious anæmia show that, in different animals, the organ for the excretion of iron may not be the same when this is greatly in excess. This fact has, to a certain extent, been illustrated also in a rutting bitch, in the intestine, liver and kidney of which I could find no evidence of excretion of iron; but sections of its uterus, after being hardened in alcohol, gave in a few seconds a marked reaction for iron confined to the mucosa. Closer examination of these resulted in showing that the iron was deposited in three situations: in leucocytes scattered in large numbers throughout the inter-glandular elements, in the cells of the long convoluted glands, and in the secretion found in the lumina of these. The leucocytes were very much enlarged, the enlargement apparently being due to the brownish masses of iron which they contained. The iron appeared to be in the phosphate form. The cytoplasm gave a reaction also for iron. In the gland cells the iron was both diffused

[1] *The Practitioner*, Vol. XLIII. 1889.
[2] *Ibid.* Vol. XLV. 1890.

through the cytoplasm and localized in the form of a row of granules immediately adjacent to the lumen of the gland. The diffuse reaction obtained was slight, but that obtained with the granules was an intense one, and the arrangement of the granules seemed to suggest that they were caused by precipitation, in an inert portion of the cell, of iron which had been in solution in the more active parts of the cytoplasm. The substance in the lumen of each gland gave a weak, but still distinct reaction for iron. Whether the leucocytes in the inter-glandular tissue carried the iron to the mucosa from other parts, or obtained it by absorption from the lymph bathing them, it was not possible to determine definitely. I could find no leucocytes in the blood vessels which contained inorganic iron compounds, and this, considered in connection with the large size of the iron masses in the inter-glandular cells, suggests that the latter derived their iron from the lymph of the inter-glandular tissue. In this case the lymph must have been also the source of the iron in the gland cells.

Why different organs in different animals should serve for the excretion of a great excess of iron, it is difficult to say. Possibly this depends on differences in the degree of activity which the organs present in different animals. It is quite as difficult to say why the kidney in some cases of pernicious anæmia and not in others should give such marked evidence, in the convoluted tubules, of the excretion of iron.

II. THE ABSORPTION OF ORGANIC IRON COMPOUNDS.

The expression "organic iron compounds" includes two chief classes of substances:—

1. The assimilated compounds of iron; that is, nuclein and related compounds, including Bunge's hæmatogen. These may be conveniently termed the "chromatins."

2. Compounds produced by a degenerative process from the first class. This includes hæmoglobin, melanin, and lardacein.

I have limited my investigation to representatives of the chromatin class.

Bunge believes that hæmatogens are formed only in vegetable life, and constitute the only compounds of iron which are absorbed by animals.

Feeding experiments with these substances have been made by Socin[1] and Busch[2]. In one experiment Socin found the amount of ingested iron exceeded that in the excreta. In the other two experi-

[1] *Zeitsch. f. Physiol. Chem.* Vol. XV., p. 93, 1891.
[2] Kobert. *Loc. cit.*

ments the reverse was the case. These experiments were made on dogs. With mice he found that iron-free food mixed with organic or inorganic iron compounds did not sustain life any longer than food free from iron, whereas control animals fed on a more natural food (coagulated egg-yolk) lived healthily. Busch from analyses of the iron in the urine after feeding on hæmatogen, hæmoglobin and hæmatin, concludes that of these hæmatogen is least absorbable. Bokay[1] found that nuclein is not digested by artificial pancreatic juice, that it leaves the body chiefly in the fæces, and causes but a slight increase in the urinary phosphates[2].

I began my own observation by feeding lake-lizards (*Necturus lateralis*) on chromatin isolated by artificial gastric digestion from lambs' testicles. The lizards were killed, and the intestines investigated by the micro-chemical methods already described. The results were negative. I then fed mice on chromatins mixed with starch and lard; they died in from six to fifteen days with symptoms of diarrhœa. They also died in a few days when the chromatin was mixed with coagulated egg-yolk, but thrived well on the egg-yolk without such admixture. I also gave to a lake-lizard the fluid from an artificial pancreatic digestion of nuclein; this fluid, however, contained little if any nuclein in solution, and micro-chemical investigation of the lizard's intestine gave negative results.

The failure of all these experiments led me to use a less abnormal kind of food; and, since, according to Miescher[3], egg-yolk itself contains 1 to 1·5 per cent. of nuclein (hæmatogen), that food substance appeared likely to yield the best results.

I used unboiled egg-yolk, for when egg-yolk is hard-boiled the yolk spherules become thereby fixed in form, and the chromatin-holding particles are set free only when the spherules are digested, but when the yolk is administered fresh the spherules readily undergo fragmentation and the chromatin-holding particles are liberated and put in a form in which the epithelial cells, if they possess the power, can invaginate them. In the spherules the chromatin is partly in a granular form[4] and, apparently, partly as envelope material to its fat globules, the latter varying in size and shading

[1] *Zeitsch. f. Physiol. Chem.* Vol. I., p. 157, 1887–8.

[2] In Gumlich's experiments, a description of which was recently published (*Zeit. für Physiol. Chemie*, Vol. XVIII., parts 5 and 6, p. 508, 1894), the absorption of nucleinic acid in the dog appeared to be shown by the increase in the amount of phosphorus excreted in the urine after the administration of a quantity of compound.—P. M. Popoff (*ibid.* p. 533) found that nuclein compounds enter into solution only in very small quantities in gastric digestion but in greater quantities as the result of the action of artificial pancreatic juice, and he points out that the occurrence of such compounds in a dissolved form is all that is necessary for their absorption in the intestinal tract.

[3] Miescher. *Hoppe-Seyler's med. chem. Unters.* Pt. 4, p. 502, 1871.

[4] Miescher. (*loc. cit.*) localized the nuclein which he discovered in egg-yolk in the granules of the yellow yolk spherules.

off into the small granules in such a way as to suggest that the latter are also fat globules of almost infinitesimal size surrounded by chromatin. Fig. 14 gives a representation of two yolk spherules which were fixed by heat and in which the iron, set free by sulphuric acid alcohol, was converted by ammonium sulphide into the sulphide. In it can be seen smaller and larger fat globules surrounded by an iron-holding envelope. The fat is, therefore, closely associated with the chromatin, and as we know the former is in some way absorbed by the intestinal epithelium, the conclusion did not appear to be a strained one that both constituents are absorbed together.

The first experiments were made with mice. The animals were isolated and fed for periods ranging from five to fifteen days with fresh egg-yolk and preparations of the small intestine were made in various ways to determine the effect. Owing to the liquid or semi-liquid form of the food the animals partook but lightly of it, and consequently there were found in the intestinal epithelium no very marked indications of absorption, even of fat. In a series of preparations from one of the animals which was allowed to live for the longer period, I found that, in the internal or lower half of each epithelial cell, the protoplasm possessed a greater capacity for staining matters like eosin than is ordinarily exercised by the same cells and, further, that this same protoplasm was denser and more finely granular. Owing to the smallness of the sections, manipulation with acid alcohols to set free the masked iron[1] to determine if this staining capacity was due to chromatin, did not meet with much success, although the teased-out cells after hardening in alcohol and long treatment with warm ammonium hydrogen sulphide and glycerine gave results which seemed to support that view. These experiments, however, indicated the direction in which my succeeding observations were to be conducted.

A large number of guinea-pigs were then fed for different periods with undiluted yolk in excess. Each animal was given about 10 c.c., three times a day, administered by the mouth by means of a glass pipette, and when it was killed the small intestine was removed and hardened, one part in alcohol and another in corrosive sublimate. The sections from the sublimate preparations made with paraffin and fixed on the cover glass by Gaule's method, were either stained with hæmatoxylin and eosin, or put through nitric acid alcohol for 8—10 hours at 35° C. and then treated with

[1] The method here referred to will be described at full length, in a forthcoming paper on the distribution of assimilated iron in animal and vegetable cells.

the acid ferrocyanide mixture to determine the distribution of "masked" iron. The alcohol material was also treated in the same way, with this exception, that the sections were fastened to the coverslip with collodion before the paraffin was removed, and when the sections were made by the celloidin process they were passed directly through nitric or sulphuric acid alcohol. The results of these experiments and methods were very interesting.

In the guinea-pig, as ordinarily fed, the "masked" iron exhibits in its distribution in the epithelium of the intestine very little difference from that represented in Fig. 6, in which the iron is shown in the chromatin of the nuclei and in a narrow zone immediately about some of the nuclei, but in preparations from animals fed with yolk for two or three days, the epithelial cells situated on the sides of the villi and below the tips of the same have the iron distributed as represented in Fig. 7, in which the whole of the protoplasm in the lower half of each cell and in the leucocytes below give a uniformly diffuse Prussian blue reaction. The epithelial cells at the tips of the villi are so much distorted by the fat present in them, that a division of each into an internal and external part is impossible, except in some cases where the absorption of fat has ceased to take place. In preparations stained with hæmatoxylin and eosin, the cells immediately below the tip give usually the appearance represented in Fig. 8, but with this exception, that the bodies enclosed in cavities of the protoplasm in the external half of each cell shown in the figure are not present in the preparations from all the animals fed with egg-yolk. The internal half of each cell is loaded with finely granular, eosinophilous material in which vacuoles are discernible, the outer half having its protoplasm arranged in a coarse meshwork, the cavities of which were probably occupied by fat globules. The nuclei in the majority of these cells contain an eosinophilous substance filling the spaces left by the chromatin network. In some villi the absorption of fat had occurred to such an extent that the epithelium was separated from the underlying tissue, as represented in the figure, and in these cases one sees sometimes a condition which explains the destiny of the finely granular, eosinophilous material in the inner ends of the cells. The corrosive sublimate has coagulated a proteid material extending from each cell and containing some eosinophilous granules. This condition I regard as due to *internal secretion* on the part of the epithelial cells, and it has its parallel in the same epithelial cells when they are transferring iron salts from themselves to the internal elements, a process which I have already described. There is a difference, however, in the two in one respect, a difference which may only be

apparent; in the internal secretion of iron, the latter appears to become dissolved before passing from the epithelial cell internally. In the yolk preparations, the eosinophilous material is in a granular form, and could one be certain that it was not due to irregular precipitation by the corrosive sublimate, it would be an indication that the epithelial cell secretes internally matters in a solid or semi-solid form. The irregular form taken by the secreted matter may be due to conditions governing the diffusion of the hardening reagent, but, from the way in which each shred of the hardened proteid secretion is arranged in its attachment to an epithelial cell, one is disposed to regard it as fixed in the condition which it occupied in the living tissue. Whether this secretion contains "masked" iron chromatin I cannot say.

Now, as in the secreting cells of all sorts, and especially in those of the Lieberkühnian glands, secretory activity is associated with the presence of a chromatin in the part of the cell remote from the lumen (Fig. 9), it might be urged that the increase of the "masked" iron in the inner ends of the superficial epithelial cells of a villus was due not to an iron compound absorbed, but to secretory activity bringing about an increase of the substance governing that process. This objection has some force, for so constantly is the presence of a chromatin (prozymogen) connected with the processes of secretion, especially in those glands which furnish a ferment, that one hesitates to deny the existence of a prozymogen in another phase of cell activity but little, if at all, different from that of secretion. The only evidence on which one can rely to show that some of the chromatin of the yolk was absorbed in this case is, that the iron reaction of the liver from an animal fed for some time with yolk is of a different character from that given by the liver of the animal after its ordinary diet. In two guinea-pigs, one fed for seven days, the other for six, when it died in a comatose condition, there was undoubted evidence of the absorption of the chromatin, for, in sections of the livers hardened in alcohol, the application of warm ammonium hydrogen sulphide for some hours gave a reaction which was most marked at the periphery of each lobule, but present also in the central zone of the same. The reaction in the central portion was more difficult to get and was best obtained when the sections were very thin. In these preparations one can determine that the iron reaction gradually becomes less intense as one follows the tissues of the lobules from the periphery to the centre. In the liver of the animal after ordinary diet the reaction with ammonium hydrogen sulphide is obtained only in the outer zone and faintly in the outer portion of the middle zone. In these, further, iron in a coarsely

granular form is almost always found in the cells at the periphery of the lobule, but such granules are absent in preparations from the animals fed with yolk for some time. In the latter the iron reaction in the liver cell is a diffuse one and it is present in the leucocytes of the capillaries as well. What seems to show that this reaction is not due to inorganic or albuminate iron, but to that in the organic form, is the length of time required to bring it out. These results have been obtained also in experiments on *Amblystomata.*

In *Amblystomata* the cellular elements, although not of the size of those in *Necturus,* are very large, and as it was possible to obtain these animals in numbers sufficient for experients in this line, I used them for this purpose. The yolk was given through a glass pipette, introduced through the mouth into the stomach, and in quantities corresponding to the size of the animal. Owing to the ease with which they may be fed, and because of the retention on the part of the stomach of what is so given, these animals are very suitable objects for experiments of this sort. Now, in those recently captured or kept in captivity for a week or so without being fed, there is comparatively little inorganic iron in the liver, no more, in fact, than there is in the liver of *Necturus* after long captivity, and in sections of the liver of such, when hardened in alcohol and treated with nitric acid alcohol, or sulphuric acid alcohol, to set free the "masked" iron, the latter is wholly confined to the nuclei of the cells. When, however, yolk is given daily in such quantities as are retained to these animals for a period of four days or more, the liver cells, which are loaded with fat, present a different reaction. When hardened in alcohol, sections of the livers give, after treatment with sulphuric acid alcohol or nitric acid alcohol for sixty hours, a reaction for iron like that shown in Fig. 13. The cytoplasm of the hepatic cell, arranged in a coarse meshwork enclosing the fat droplets, carries in its trabeculæ irregularly shaped masses of iron-holding substance which are sometimes extended along and in the trabeculæ. The nucleus gives the usual reaction for "masked" iron. The iron in the cytoplasm is of the "masked" character, for isolated cells mounted with a mixture of ammonium hydrogen sulphide and glycerine, and kept at a temperature of 60°C., gave an iron reaction in the masses in the cytoplastic trabeculæ only after three days, which became more marked on the fifth day. The nuclei in these cells gave but a feeble reaction for iron, owing, apparently, to the fact that the "masked" iron in the cytoplasm had used up all the decomposing energy of the sulphide and therefore, apparently, little of the active reagent reached the nuclei. That the iron in the masses of the cytoplasm is not due to the dif-

fusion from the nucleus, was shown by the non-occurrence of such iron compounds in the cytoplasm of hepatic cells from *Amblystomata* recently captured, or kept in captivity without food. In those *Amblystomata*, in the intestine of which on capture, food matters, such as mollusca, worms and insects, were found, the cytoplasm of the hepatic cells gave no reaction for "masked" iron. The difference in this respect between the liver cells of the recently captured or fasting animal, and those of the animal which had been fed for several days with egg-yolk, was readily shown by putting thin sections of the organs, hardened in alcohol, taken from animals in the two conditions, into the same dish of sulphuric acid alcohol for three days. At the end of this time, treatment of the two kinds of sections with the acid ferrocyanide mixture gave different results in both cases. In both the nuclei exhibited an equally intense iron reaction, but in the cytoplasm of the cells from the fed animals, the masses of the trabeculæ were as distinctly iron-holding as in the cells treated with ammonium hydrogen sulphide, while the cells from the livers of the other animals exhibited no such reaction.

This subjection of sections to treatment under exactly the same conditions, considered in connection with the results obtained by using ammonium hydrogen sulphide, shows that the iron found in the masses situated in the cytoplasmic trabeculæ was due to a "masked" combination there placed. That it was a chromatin was shown by its capacity for taking up staining matters. In sections of the liver in this case, when hardened either by the alcohol or corrosive sublimate method and stained with hæmatoxylin, safranin, or eosin, the cytoplasmic masses were colored, though not as deeply and intensely as the nuclear elements, yet as distinctly as the latter. In sections from the liver of the unfed or recently captured animal, when similarly treated, there was a complete absence of such stainable material in the cytoplasm. When sections of the liver from the fed animal were treated with sulphuric acid alcohol to set the "masked" iron free and subjected to the Prussian blue reaction, subsequent staining with safranin gave a violet-like combination of the blue and the safranin red in the chromatin of both the cell and the nucleus.

That the cytoplasmic chromatin in this case comes from the chromatin of the yolk, and is not due to a prozymogen called into existence by the abnormal quantity of food matter in the hepatic cell, I tried to show by feeding *Amblystomata* with olive oil. In those fed with yolk the hepatic cells were very greatly loaded with fat. A similar excess of fat in the liver cells was obtained by feeding with oil, but in these there was no cytoplasmic chromatin.

In thin sections from the intestine of the *Amblystoma* fed on egg-yolk, the presence of "masked" iron compounds in the cytoplasm of the epithelial cells was not indicated readily, owing to the abundance of fat in the same. The more active the absorption of the yolk elements had been, the less, therefore, was it possible to demonstrate the existence of cytoplasmic chromatin. I have found, however, as in the intestinal epithelium of guinea-pigs after they are fed on yolk for some time, that the inner portion of each epithelial cell not overloaded with fat, was filled with an eosinophilous, finely granular material from which sulphuric acid alcohol sets free iron. I found also that if an *Amblystoma* is fed for several days with yolk and then allowed to fast for several days, in its intestinal epithelium now freed from fat there is, as shown by sulphuric acid alcohol, a slight amount of "masked" iron diffused through the cytoplasm. Whether this is derived from the yolk, or is that of a prozymogen, it is as difficult to decide as in the case of the intestinal cells of the guinea-pig under similar conditions.

There is a difference between the chromatin of the yolk and that of the hepatic cytoplasm of an *Amblystoma* fed with yolk, which is probably to be explained as due to a chemical transformation effected by the absorbing cells or by the liver cells. According to Bunge[1] the chromatin of egg-yolk, or hæmatogen, as he terms it, when isolated, gives no immediate reaction with ammonium sulphide, but after the lapse of half-an-hour, a slight green color appears, which, in the course of several hours, deepens to an intense dark green, and several days after becomes black and opaque, the various appearances being hastened by increasing the volume of ammonium sulphide added. I find that the addition of ammonium hydrogen sulphide to fresh yolk, calls forth in two or three minutes at most the fullest iron reaction of which it is capable. The difference in this respect between isolated and unisolated yolk chromatin, is to be explained by the finer division and distribution of the latter in the spherules which the reagent disintegrates and partially dissolves before attacking the chromatin, and by the consequently readier action of the sulphide. When, on the other hand, the spherules have been fixed by heat or by alcohol before subjection to ammonium hydrogen sulphide, the iron reaction is almost as slowly obtained as in the case of the isolated yolk chromatin, the slowness of the reaction being partly caused by the difficulty with which the sulphide penetrates the spherules. In the livers of the *Amblystomata* fed with yolk the cytoplasm chromatin is readily

[1] *Loc. cit.* I have elsewhere criticised Bunge's description of this compound and have pointed out some mistakes into which he has fallen.

reached by the ammonium hydrogen sulphide, but it takes several days to give the iron reaction, this indicating that the iron there is more firmly held than it is in the yolk. This difference corresponds to that found in the yolk as it is being assimilated in the developing larvæ of *Amblystoma*. In these the yolk chromatin is in form of homogenous spherules which, when fresh or hardened in alcohol, give, in a minute or two after treatment with ammonium hydrogen sulphide, a dark green reaction for iron, also shown, though less readily, by the nuclei of the cells containing the spherules. Until the spherules are completely dissolved by the containing cells the nuclei give this reaction, but, when they have disappeared, then the iron reaction in the nuclei is obtained with as great a difficulty as it is in those of the adult animals. In other words, the yolk chromatin, as it is assimilated, undergoes a change whereby the iron in it is more firmly fixed or more "masked." It is extremely probable that the difference between chromatin of hen's egg and that of the hepatic cytoplasm of *Amblystomata* fed with it, is to be explained in the same way[1].

The yolk of old or stale eggs gives, with ammonium sulphide, the iron reaction almost immediately, and is, in this respect, more suitable than the fresh yolk to determine if the absorption of yolk chromatin obtains. I made two experiments with it, one with a guinea-pig kept without food for seven days, the other with a lake-lizard which had been kept without food for over two years.

The guinea-pig was killed five hours after it was given 12 c.c. of stale yolk, and parts of the small intestine and the liver were removed and put into alcohol. Pieces of both organs were, in the fresh condition, teased out on the slide in a drop of ammonium hydrogen sulphide and immediately examined under the microscope. The preparations from the liver yielded nothing distinctly demonstrating that absorption had taken place, but in the tips of the villi in which fat absorption was prominently shown, there was a dull green color apparently confined to the sub-epithelial elements. The fat-holding epithelial cells of the fresh villi were easily removed in some instances by teasing, and in some of these denuded villi

[1] Some change takes place in the iron-holding substance in the cytoplasm of the hepatic cell of *Amblystoma*, as shown by its staining reaction. In the animals fed with egg-yolk for some time, the hepatic cells, when hardened with alcohol or corrosive sublimate and stained with hæmatoxylin and eosin, were found to contain, in that part next the blood capillary, eosinophilous masses and in that part adjacent to the bile capillary masses which had a violet stain. Both species of contents were formed of an organic iron compound and between both there were transition elements in which the affinity for hæmatoxylin was as great as that for eosin. It would appear as if the eosinophilous substance which, judging from its position, is possibly derived by the cell from the blood, becomes converted by the cell into that substance which manifests a greater affinity for hæmatoxylin.

appearances were obtained with ammonium hydrogen sulphide which resembled those represented in Fig. 10. Here greenish granules were found in the adenoid tissue and the leucocytes which, in some instances, were collected in groups immediately under the epithelial layer. Halfway down the villus the greenish reaction became indistinct. In sections of the villi hardened in alcohol, the ammonium hydrogen sulphide gave a green reaction most frequently confined to a zone immediately below the epithelium of the tips of the villi, but sometimes extending into the lower ends of the epithelial cells themselves. The reaction of the sub-epithelial zone was chiefly in the leucocytes, and in these the iron was rarely in the granular form observed in the fasting animal or in one fed on ordinary diet. This difference indicates that the iron in these cases is derived from different sources. The difference between the disposition of the iron in the fresh villus, and that found in the villus hardened in alcohol, is to be attributed to the ammonium hydrogen sulphide altering the arrangement and character of the sub-epithelial elements when these were fresh, and to the extraction by the alcohol of the fat droplets and globules in the leucocytes which, consequently, were more uniformly colored by the reagent than was the case with the fresh elements.

There was evidence of the transference of yolk chromatin from the epithelial cells to the underlying elements, and this was found in those villi in which the process of fat absorption had not distorted the cells. Sections of these villi, obtained from material hardened in alcohol, when treated with ammonium hydrogen sulphide, gave preparations like that of which Fig. 12 is an illustration. The inner portions of some of the cells at the extreme tip of a villus gave a faint greenish reaction immediately after the reagent was added, but in the corresponding portions of other epithelial cells the reaction was given also by granular elements lying between and among the fat droplets. When the cell was loaded with fat the greenish reaction was not obtainable. The underlying leucocytes appeared, frequently, placed against the lower ends of the epithelial cells, and when this was the case the greenish granules in the latter were accompanied by similar elements in the more superficially placed leucocytes. The iron compound in the lower portions of the epithelial cells differed, in the readiness with which it reacts on the addition of the sulphide, from that found in the same structures when fresh yolk was fed, as in the earlier experiments.

The lake-lizard, five hours after 4 c.c. of stale yolk was injected into its intestine, was killed and the intestine removed, cut open and put in alcohol. Sections, as well as teased-out portions of the

hardened mucosa, were examined when mounted in a mixture of the sulphide and glycerine, both before and after the application of warmth. The results of this experiment were not as decisive as those of the last described, for the reason, apparently, that as the digestive and absorptive powers had been so long unused, a longer time than five hours for the absorption of the yolk should have been allowed. The yolk had mingled with mucus and in many places formed with it a coagulum which, even in the fresh state, came away leaving the surface of the mucosa perfectly clean. In other parts, chiefly at the tips of the longitudinal folds, fat absorption had occurred, causing an extraordinary distortion of the epithelial cells and amongst these, shaped and moulded by the fat-loaded cells, were leucocytes which also held fat and at the same time gave a very strong iron reaction with ammonium hydrogen sulphide. Below these points there were large collections of leucocytes with scarcely less iron than had those amongst the fat-holding epithelial cells. Leucocytes were also found in the tips of other folds projecting beyond the free border between fat-holding epithelial cells, the part projecting being surrounded by yolk and containing both fat and iron compounds. That a portion of this iron was of the organic form was shown by the fact that the reaction first obtained with ammonium hydrogen sulphide became deeper when the preparation was kept for an hour at 60°C. It of course does not follow that this was derived from the yolk, but that they received a part of it from this source, appeared to be indicated by the greater abundance of iron-holding leucocytes under those tips of which the epithelial cells were loaded with fat.

How the chromatin is transferred to the liver I cannot say definitely, but the capacity of the leucocytes for carrying inorganic compounds of iron and the occurrence of iron-holding leucocytes in the capillaries of the liver of an *Amblystoma* fed for a time with yolk, gives a basis for the opinion that they are the chromatin carriers. Furthermore, we know that in the performance of their function as phagocytes they invaginate and dissolve the remains of disintegrated cells, and there is very little apparent difference between this and that of taking up particles, holding chromatin, which may reach them through the process of internal secretion already described. This view would explain the occurrence of leucocytes which gave the diffuse iron reaction in the villi of guinea-pigs fed with stale yolk.

There remains another question: How does the chromatin of the yolk gain access to the interior of the epithelial cells? Upon this point I have not much to offer, although I have often en-

deavored to determine the mode of entrance. The intimate relations of the fat and the chromatin in the yolk suggest that the method of absorption of both is the same, that is, that when the epithelial cell takes in fat, it also receives the chromatin which appears to form envelopes for the most minute fat particles in the yellow yolk spherule. The chromatin of yolk would, when deprived of its intimate association with fat, therefore, be unabsorbable, even though mechanically mixed with a quantity of the same. This would explain some of the results of Sucin's experiments already quoted. That investigator fed mice with pure hæmatogen (of yolk) mixed with the proteids of serum, hog's fat, and starch, and found that the animals did not live as long as those fed on absolutely iron-free food, while those fed on hard-boiled egg-yolk mixed with starch and cellulose lived and throve on the diet. If the fat of yolk assists in the absorption of its chromatin, then yolk from which the fat has been extracted with ether, mixed with hog's fat and starch, ought to give the same result when fed to mice as was obtained in Socin's experiments with pure hæmetogen mixed with other food elements. The result of such an experiment would indeed be interesting.

As already mentioned, I have made frequent attempts to determine how the particles of chromatin enter the epithelial cells. I have in all of these used alcohol material, which was teased out on the slide and mounted in glycerine and ammonium hydrogen sulphide. The idea with which I made these attempts was that the chromatin particles, rendered greenish by their iron reaction, ought to be easily seen with careful adjustment of light and the best optical appliances obtainable. Of all these endeavors but one seemed to give good results. This was furnished by the epithelium of an *Ambiystoma* which had, 24 hours before being killed, been artificially fed with yolk. The appearances to be described were found in isolated spots only on the tips of the longitudinal folds in the intestine. The free margin of the cell was covered with granular matter from the yolk, in which one could see very minute fat globules intermingled with particles having a greenish color. The line of separation of this material from the striated border was, in the majority of the few cases observed, not distinct. The striated border did not give the usual appearance, the striæ being apparently replaced, each by a row of oval vesicles with greenish envelopes. With 1·5 mm. apochromatic immersion (Zeiss) and compensation ocular 4, the vesicles could be seen connected by a grayish line. On the protoplasmic side of the margin were also minute vesicles with greenish envelopes, apparently of the same character as those

in the striated border and the reticulated protoplasm itself had a slightly greenish tinge (Fig. 15). Whether the oval vesicles in the margin were in the rodlets ("stäbchen" of the Germans) or between the same it is not possible to say, for in this case one deals with structures which, in their size and optical properties, approach the limits of microscopic vision. It was also impossible to determine whether these vesicles were being invaginated by the epithelial cells or were mechanically entangled by the rodlets. It may, in fact, be that the oval vesicles were not connected with absorption at all and that they were merely appearances in the rodlets, although, on this view, the green reaction in their envelopes would be difficult to explain. That the rodlets are not always simple structureless elements, I have, several times, found to be the case in preparations from the guinea-pig (Fig. 11), in which each rodlet appeared to be a series of beadlets or granules. These may have been produced by the reagent used, but their occurrence indicates that the rodlets are not homogeneous elements. Heidenhain[1] observed in the salamander, the dog and the cat, a thickening of the lower end of each rodlet. In my preparations the basal granule of each rodlet was most distinct.

It does not appear that the absorption of chromatin and fat goes on in the proportion in which they occur in yolk. In sections, made by the celloidin method, of the intestinal mucosa and its adherent yolk, from an *Amblystoma* fed with yolk, I found above and resting upon the epithelium, here and there, homogenous clumps of chromatin which are not found in the yolk in its ordinary condition. When these sections are for time kept in contact with ammonium hydrogen sulphide at 60° C., the clumps give a decided iron reaction. The occurrence may be explained by the existence, on the part of the epithelial cell, of a capacity for the absorption of fat greater than that for the absorption of chromatin.

I have already referred to the occurrence of bodies in the outer halves of the intestinal epithelial cells in preparations from a guinea-pig which had been fed with yolk for thirty-six hours. These elements are not iron-holding, as they do not yield any iron reaction after treatment for days with ammonium hydrogen sulphide, or after treatment with acid alcohols. They possess, however, a greater affinity for dyes like eosin and aurantia than the surrounding cytoplasm, and solutions of Ehrlich's hæmatoxylin give them a feeble violet color. Like nuclear chromatin they absorb and tenaciously hold mineral reagents, as, for example, ammonium molybdate[2] and this fact, considered in connection with their capacity for

[1] *Archiv für d. ges. Physiol.* Vol. LXIII. Supplement, 1888.

[2] As employed by Lilienfeld and Monti to determine micro-chemically the presence of phosphorus (*Zeit. für Physiol. Chemie.* Vol. XVII. p. 410).

absorbing dyes, would seem to suggest that they belong to the nuclein class of compounds. Somewhat similar bodies have been observed by Heidenhain[1] in the epithelial cells of the intestinal villi in a new-born pup which had suckled, and he has figures also, in one of the plates accompanying his paper, of a portion of a villus from a rabbit after being fed with milk, in the epithelial of which are shown a number of similar bodies. He found their size and number to vary in different cells, while they were absent in the fœtus and in a pup twelve days old. He regarded them as albuminous excretions from the protoplasm which appear at the commencement of the absorptive process, but gradually vanish. If the bodies which I find in my preparations are of the same nature as those described by Heidenhain, a different explanation of their origin appears necessary. It is remarkable that they should occur after the commencement of milk or yolk feeding, and as both foods contain proteids intimately associated with the fat globules, the absorption of the proteids, as well as of the fats, must entail upon the cells the disposal of some of the former which are at first physiologically cumbersome as it were, and which, possibly, are disposed of temporarily in the form of these protoplasmic masses. The casein of milk and the vitellin of yolk, both phosphorus-holding compounds, may thus contribute to their formation. The only difficulty experienced in accepting this view of their origin is that it does not explain, in the results obtained with a yolk diet, why these elements are absent where the yolk absorption goes on most vigorously, that is, in the cells at the extreme tips of the villi, while they are present in those cells in which yolk absorption is less active, and why also these elements are absent after two days of a yolk diet. Further investigation is necessary upon all these points.

SUMMARY.

1. The experiments on the administration of inorganic compounds of iron to guinea-pigs and other animals have resulted in showing that the intestinal mucosa absorbs these to an extent which varies with the nature of the compound and with the quantity of it given. When the dose is small, absorption occurs only in that part of the intestine adjacent to the pylorus and measuring only a few inches in length, yet when the quantity given at any one time is large, the absorptive area may embrace the whole of the small intestine. In the former case the result appears to depend on the complete precipitation, as hydroxide, of the iron of the salt unabsorbed, in the thoroughly mixed bile, chyme, and pancreatic juice; and in

[1] *Loc. cit.*

the latter case the large amount of the iron salt, apparently, first destroys the alkalinity of these fluids, the excess of the salt unaffected and remaining in solution then undergoing absorption.

2. The intestinal epithelial cells transfer the absorbed iron at once to the underlying elements when the quantity absorbed is small, but, with a large amount absorbed, the epithelial cells are found to contain some of it.

3. Though some of the sub-epithelial leucocytes of the villi appear to carry part of the absorbed iron into the general blood circulation, probably the more important agent in the transference of the inorganic iron from the villi to other parts of the body is the blood-plasma.

4. Marfori's albuminate and the commercial "peptone" of iron, when administered to guinea-pigs, seem to stimulate the leucocytes to invade the epithelial layer of the intestinal villi.

5. Of the organic iron compounds belonging to the "chromatin" class, that present in egg-yolk (hæmatogen of Bunge) undergoes absorption in the intestine of the guinea-pig and of *Amblystoma*. In these, but more especially in the latter, after they are fed with egg-yolk for several days, the cytoplasm of the liver cells yields marked evidence of the presence of an organic iron compound belonging to the "chromatin" class and derived from the yolk administered.

6. The mode of absorption of yolk "chromatin" is obscure, but the process appears, in some way, to be connected with the absorption of the fat with which the iron compound is closely associated in yolk.

EXPLANATION OF FIGURES*.

PLATE XI.

NOTE. In the preparation of all the figures (except 15) Abbe's camera was employed, and all, with the exception of two, are illustrated as they were seen with an immersion apochromatic objective (Zeiss 3 mm., 2 mm. or 1.5 mm. focus). The exceptions are Figs. 1 and 2, in the drawing of which Zeiss D. was used.

Fig. 1. Section of a villus from the pyloric end of the small intestines of a guinea-pig kept on ordinary diet. Alcohol, acid ferrocyanide mixture, balsam. x 305.

Fig. 3. A portion of the mucosa of the intestine in a lake-lizard, "e," epithelial cells, "l," iron-carrying leucocytes, "r," red blood corpuscles, also shown to contain inorganic iron. Alcohol, acid ferrocyanide mixture, balsam. x 620.

Fig. 4. A portion of the epithelium and underlying elements

*Taken from the Journal of Physiology, April, 1894; p. 295.

of an intestinal villus of a guinea-pig after the administration of "peptonate" of iron. "L," leucocyte, "b c," blood capillary. Alcohol acid ferrocyanide mixture, balsam. x 1240. Drawn with the diaphragm of Abbe's condenser removed from the microscope.

Fig. 5. Portion of the section of the liver of the guinea-pig fed with "peptonate of iron. "L," leucocytes, "h c," hepatic cells, "b c," blood capillary. x 1240. Drawn with the diaphragm of the condenser removed.

Fig. 6. A portion of the tip of an intestinal villus oi a guinea-pig kept on its ordinary diet, to show the distribution in the cells of the organic iron compounds (chromatins). "E," epithelial cells, "l," leucocytes, "a," neuclei of adenoid elements. In the cytoplasm of two of the leucocytes are found granules of an inorganic (?) iron com pound. Alcohol, nitric acid alcohol, acid ferrocyanide mixture, balsam. x 1240. Drawn with the diaphragm of the condenser removed.

Fig. 7. Epithelium and underlying elements from the side of a villus of a guinea-pig on the second day of the course of yolk-feeding, to show the distribution of organic iron compounds (chromatins) "L," leucocytes, "a," the sub-epithelial "membrane." Alcohol, nitric acid alcohol, acid ferrocyanide mixture, balsam. x 1240. Drawn with the diaphragm of the condenser removed.

Fig. 8. Epithelium and underlying elements of a villus of the same animal. "A," sub-epithelial "membrane," "s," the secretion from epithelial cells. Corrosive sublimate, hæmatoxylin, eosin, balsam. x 1240.

Fig. 9. Portion of a Lieberkühnian gland of a guinea-pig to show the distribution of organic compounds of iron (Chromatins) and especially of those connected with secretion. Alcohol, nitric acid alcohol, acid ferrocyanide mixture, balsam. x 620. Drawn with the diaphragm of the condenser removed.

Fig. 10. Portion of a fresh villus of a guinea-pig killed five hours after being fed with stale yolk. The epithelium has been removed. Ammonium hydrogen sulphide, glycerine. x 920.

Fig. 11. Intestinal epithelial cell of a villus from the same animal. Alcohol, ammonium hydrogen sulphide, glycerine. x 820. (Zeiss oc. 4, apochr. imm. 1.5 mm.)

Fig. 12. Epithelium and underlying leucocytes of a villus from the same animal, to show the absorption of the yolk chromatin. Alcohol, ammonium hydrogen sulphide, glycerine. x 620.

Fig. 13. A liver cell of an Amblystoma fed artificially for four days with egg-yolk. Alcohol, sulphuric acid alcohol, acid ferrocyanide mixture, balsam. x 820. Drawn with the diaphragm of

the condenser removed.

Fig. 14. Yolk spherules from a hard-boiled egg, to show the distribution of the iron (of the hæmatogen). In the spherule on the left the elements are fewer and coarser. Sulphuric acid alcohol (for 48 hours), ammonium hydrogen sulphide, glycerine. x 820. (Comp. oc. 4, imm. apochr. 1.5 mm.)

Fig. 15. Free border of an intestinal epithelial cell of an Amblystoma fed with yolk. "P," cell protoplasm, "h," hyaline border, "y," elements of a yolk. Alcohol, ammonium hydrogen sulphide and glycerine (at 60 c. for eight hours). (Comp. oc. 4, apochr. 1.5 mm.)

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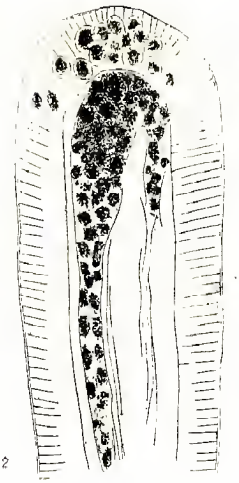

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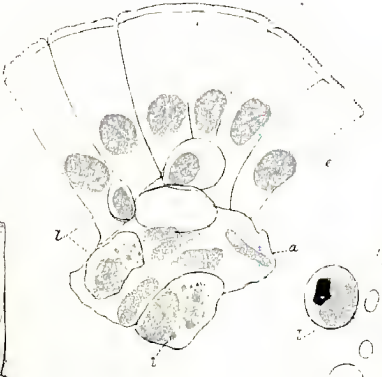

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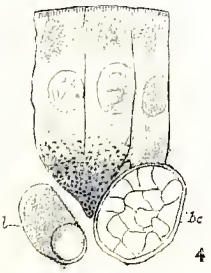

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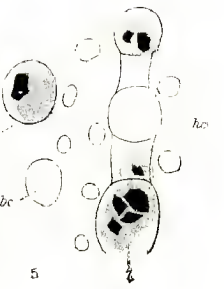

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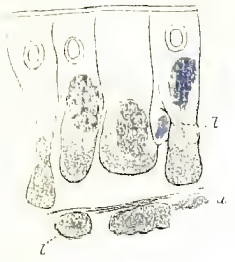

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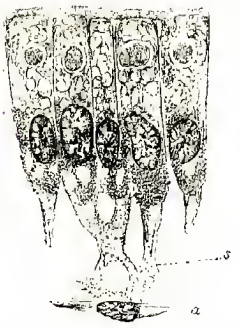

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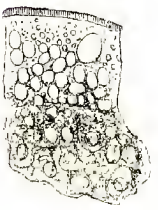

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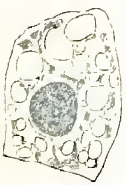

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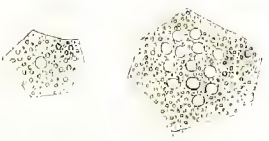

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Edwin Wilson Cambridge

PART III.

The Hæmoglobins and Related Products of the Market.

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THE HÆMOGLOBINS AND RELATED PRODUCTS OF THE MARKET.[1]

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The Hæmoglobins of the market are comparative innovations. Side by side with ferric tablet triturates, troches, etc., the Hæmoglobins have come, adding their quota to the elegance of medicinal ingestion.

It is well known that Hæmoglobin contains iron, naturally combined as it exists in the normal blood. Physiologists disagree as to the absorption and disposition of iron when taken into the system. "The iron salts," says Bunge, "seem not to be absorbed at all in the intestinal canal, or only to such a small extent that it is questionable whether their absorption has any mentionable importance." "It seems more probable that the absorption of iron from the food takes place in the form of proteid bodies containing iron." The only importance that Bunge attributes to the ingestion of these bodies is that they unite with the hydrogen sulphide of the intestine to form iron sulphide, thus conserving for absorption the iron which the food contains.[2]

If nature thus provides sufficient iron in the food we eat, why give iron at all? Why not administer the salts of some other metal, such as manganese or bismuth, which would with equal readiness unite with the intestinal gases? In chlorosis, which is pre-eminently the result of a lack of iron, the salts of manganese and bismuth have been substituted, but the disease will not yield to them. This fact certainly offers some evidence in support of the theory that iron plays some other part aside from mere sulphide combination and ejection. Physiologists have told us that the soluble forms of iron are readily absorbed and unite with the albumen of the blood to form albuminates, and that the insoluble preparations do likewise after having been changed into chlorides by the gastric juice.

[1] From the Pharmaceutische Rundschau, September, 1894.
[2] See also Pharm. Rundschau, vol. 9, p. 114, and vol. 10, p. 267.

The administration of iron as it exists in *Hæmoglobin*, seems to be based upon a combination of the above rival ideas; for according to Bunge, absorption only occurs in the case of proteid bodies containing iron. Hæmoglobin is pre-eminently a proteid body, and if iron must eventually become an albuminate and exist as such, then its ingestion as an albuminate removes the burden of an intermediate formation of a chloride, for gastric digestion does not form a chloride of the natural proteid iron, but rather converts it into acid-hæmatin. Hammerston, in his physiological chemistry, says, "Hæmatin is formed by the action of the gastric juice upon oxyhæmoglobin." The writer's own experiments confirm this statement, for, of the six proteid irons of the market which were subjected to gastric digestion, all but one were so converted. That one was not a natural proteid compound, but a synthetical product. The hæmatin so formed is said by Hammerston to be changed into bilirubin by the liver; this change necessitates the setting free of the combined iron. The question naturally arises, "is the iron entirely eliminated by the bile?" Kunkel says not; for "in 100 parts of bilirubin, eliminated by the bile, there are only 1.4 to 1.5 parts of iron; while 100 parts of hæmatin contain about 9 parts of iron."

The terms *Hæmoglobin* and *Oxyhæmoglobin* are often used interchangeably; whereas oxyhæmoglobin is a molecular combination of the former with oxygen; the oxygen is loosely combined and easily disassociated. Arterial blood is nearly all Oxyhæmoglobin, with some Hæmoglobin, while venous blood contains large quantities of the latter. Methæmoglobin is a transformation product of Oxyhæmoglobin, while Hæmatin is a decomposition product. Characteristic absorption bands are given by each of the above substances with the spectroscope and are of great importance in their identification.[1] Normal, freshly. drawn blood, which is mostly Oxyhæmoglobin, shows two absorption bands between the lines D and E. Band *a* is narrower but darker and more clearly outlined, and lies on the line D. Band *b* is broader, less dark and less defined and lies at E. Hæmoglobin readily absorbs oxygen from the atmosphere and is converted into Oxyhæmoglobin; its spectrum, however, shows a broad not clearly defined band between D and E. Methæmoglobin has the same composition as Oxyhæmoglobin. If arterial blood be sealed in a tube, it gradually consumes its oxygen and becomes venous, and by this absorption of oxygen a little methæmoglobin is formed. Methæmoglobin also presents two absorption bands; but band *b* is stronger and more definitely outlined than band

[1] See PHARM. RUNDSCHAU, vol. 10, pp. 56—59.

a; besides this difference from Oxyhæmoglobin, the bands are more toward the red part of the spectrum. Hæmatin, in acid solution, absorbs more of the violet than of the red. It shows a sharply defined band between *c* and *d*, and a broader but less clearly distinct band between *d* and *f*. On diluting the acid-hæmatin solution, four bands manifest themselves; the broad one between *d* and *f* dividing into two, while a very indistinct band appears on the *e* side of *d*. The Hæmoglobins examined by the writer were subjected to spectroscopic analysis and the above absorption bands determined.

What chlorophyl is to the leaf, Hæmoglobin is to the blood, namely: its coloring substance. Iron is an integral constituent of this coloring substance and its function is to take up oxygen. Its presence, therefore, is of vital importance. It necessarily follows that an insufficient supply of iron causes an inadequate formation of Hæmoglobin. As Hæmoglobin, after having been oxidized into Oxyhæmoglobin in the lungs, is the oxygen carrier of the blood, the sequence of this blood poverty is lessened tissue oxidation.

Before beginning experimental work upon the Hæmoglobins and their related products of the market, Hæmoglobin was prepared. The method employed was that recommended by Hoppe-Seyler. Sheep's blood was used. This was defibrinated immediately upon withdrawal from the body. In the absence of a centrifugal machine the defibrinated blood was allowed to settle and the serum separated from the corpuscles. These were put into three volumes of water and heated to 35° C. The temperature was then reduced to 0° C. and the mixture washed with ether and this was afterward replaced with one-fourth its volume of alcohol. From this alcohol solution, Hæmoglobin separated, after having been placed in a freezing mixture during 24 hours. The Hæmoglobin thus obtained was dried *in vacuo* upon an absorbing plate over sulphuric acid. It occurred as an amorphous powder, reddish-brown in color, bloody odor and albuminous taste. The spectroscope revealed it to be a mixture of Oxyhæmoglobin and Methæmoglobin.

Similar experiments were made upon the blood of the ox; also upon the blood of the cat. Repeated attempts upon the ox-blood failed of any satisfactory results in the formation of crystals. From the cat's blood, crystals of Oxyhæmoglobin were obtained.

The Hæmoglobins of the market have been examined with respect to external form, color, ódor, taste, solubility, reaction, percentage of iron, artificial gastric digestion and composition. The percentage of iron obtained in each case is not the result of merely one estimation, but of several, and in some cases, estimations from different samples.

The more recent proteid irons of the market which have come to notice are the following: "a" *Hæmoglobin* (Merck); "b" *Hæmoferrum;* "c" *Ferroglobin;* "d" *Ferrum Sanguinis* (Chapoteaut); "e" *Ferratin;* "f" *Hæmogallol;* "g" *Desiccated Defibrinated Blood;* "h" *Hæmoglobin Compound.* These will be considered in the order named.

a) *Hæmoglobin* from Merck, occurs in fine, chocolate brown, amorphous powder. The odor is somewhat putrid, the taste albuminous. It is very slowly soluble in water and leaves considerable residue. Its reaction is neutral. Several estimations were made for iron on two different samples, one purchased in Detroit and the other in New York. The result shows .504 per cent. of metallic iron. Upon subjection to artificial digestion, it was converted into acid Hæmatin. The spectroscope reveals it to be a mixture of about equal parts of Oxyhaemoglobin and Methaemoglobin. It possesses the common property with other Haemoglobins of burning with an empyreumatic odor. Artificial digestion with this sample, as with the others, was conducted in a pepsin tester, kept between 37° to 40° C. and subjected to constant agitation. With this Haemoglobin, as with the remaining proteids, the iron estimation was conducted volumetrically. The Haemoglobin was first incinerated in a platinum crucible. The ash was then dissolved in hydrochloric acid, afterward replaced by sulphuric acid. The resulting iron salt was then completely reduced by nascent hydrogen to a ferrous condition; this was then titrated with $^{N}/_{50}$ potassium permanganate.

b) *Haemoferrum* comes upon the market in three-grain gelatin pilloids, inclosing a "pseudo-crystalline" powder. The color is reddish-brown, while the odor is like that of blood. The taste is simply albuminous. It is almost entirely soluble in water and has a neutral reaction. Iron estimation shows .411 per cent. Artificial gastric digestion converts it into acid-haematin. It is mostly Oxyhaemoglobin, but shows traces of Methaemoglobin.

c) *Ferroglobin* occurs in two-grain, compressed, sugar-coated tablets. The color is reddish-brown, the odor that of blood. The taste resembles that of burnt albumen. It is composed of a soluble portion and an insoluble portion. The soluble portion is all Oxyhaemoglobin, and constitutes about one-third of the powder, while the insoluble portion is Haematin. Titration revealed .299 per cent. of iron.

d) *Ferrum Sanguinis* is in gelatine globules inclosing minute, brownish-black scales. The odor is putrid, the taste albuminous, and upon dissolving in water leaves a large albuminous deposit. Its reaction is neutral. Iron is present to the amount of .418 per

cent. Is converted into acid-haematin by digestion. The spectrum shows it to be mostly Methaemoglobin with a trace of Oxyhaemoglobin,

e) *Ferratin* is not a natural proteid form of iron, but a synthetic product. It "is artificially prepared from egg albumen and chemically pure iron salts in alkaline solution." It occurs as a very fine, gritty, cinnamon-brown powder; the odor is somewhat alcoholic at times; when confined, it resembles that of brown sugar. The taste is somewhat ferruginous. It is insoluble in water but soluble in a faintly alkaline mixture to a reddish-brown solution. Several titrations on different samples show the amount of iron to be 5.707 per cent. It shows no absorption bands, while artificial digestion converts its iron into a chloride.

f) *Haemogallol* is made by the action of pyrogallol upon Haemoglobin. It is a fine, slightly gritty, snuff-brown powder, with an ethereal-alcoholic odor and a slight ferruginous taste. It is insoluble in water, but soluble in alkaline mixture to a greenish-yellow solution. It shows no absorption bands and digestion converts it into acid-haematin. Its percentage of iron is .444%. *Haemol*, a proteid iron, is closely allied to Haemogallol. It is obtained similarly to Haemogallol; the only difference in its manufacture being the use of zinc dust instead of pyrogallol.

g) *Desiccated Defibrinated Blood* occurs in long brownish black scales. Its odor is putrid, its taste is albuminous, and it is very slowly soluble in water. If the scales be placed into hot water, they swell and become plastic and gelatinous. The reaction is neutral; the amount of iron .149 per cent. Its spectrum shows Methaemoglobin with a trace of Oxyhaemoglobin.

h) *Haemoglobin Compound* is a brownish black liquid. The odor is that of burnt malt, while the taste is sweet, alcoholic and malty. It is readily miscible in water and has an acid reaction. It contains .015 per cent. of iron. Its spectrum shows it to contain a mixture of Oxyhaemoglobin and Methaemoglobin.

Considerable evidence is at hand of the efficacy of the Haemoglobin treatment. Especially so from hospital service. Dr. LeBon in speaking of the use of Haemoglobins, says,—"desiccated ox blood and Haemoglobin has been thoroughly tried in the hospitals of Paris and found very efficacious in treating debilitated patients." Also Benczür (*Disch. Med. Ztg.*) reports upon the Haemoglobin treatment as carried on in von Liemssen's clinic. "Troches were prepared from ox blood. The daily amount of Haemoglobin given was about 25 grains. Not only was there marked improvement in the cases of anæmia thus treated but no gastric disturbance was observed."

TABLE SHOWING COMPARATIVE TESTS.

	External Form.	Color.	Odor.	Taste.	Solubility.	Reaction.	Per Cent of Iron.	Gastric Digestion.	Composition
Haemoglobin (Merck)	Fine powder.	Chocolate Brown	Putrid	Albuminous.	Slowly sol. Leaves considerable residue	Neutral	.504	"Acid" Haematin.	Methaemoglobin. Oxybaemoglobin equal parts.
Hæmoferrum	3 grain gel. pilloids "Pseudo-crystalline" powder.	Reddish Brown	Blood	"	Nearly all soluble	"	.411	"	Mostly oxybaemoglobin. Traces of Metbaemoglobin.
Ferroglobin	2 grain compressed sugar coated tablets.	"	"	Burnt Albumen	Partly soluble	"	.299	"	Oxyhaemogl. in the sol. portion. Haematin in the insol. portion.
Ferrum Sanguinis (Chapoteaut.)	Gelatine globules enclosing minute scales	Brownisb Black	Putrid	Albuminous	Leaves a large albuminous deposit	"	.418	"	Mostly Metbaemoglobin. Traces of oxyhaemoglobin.
Ferratin	Fine powder	Cinnamon Brown	Ethereal-alcoholic	Ferruginous	Sol. in faint alk. sol. to reddisb-brown sol.	"	5.707	Chloride	No absorpt. bands
Haemogallol	"	Snuff Brown	"	Slightly Ferruginous, gritty	Slowly in alk. sol. to greenish-yellow sol.	"	.444	Acid Haematin.	"
Desiccated Defibrinated Blood	Large scales	Brownish Black	Putrid	Albuminous	Very slowly soluble	"	.194	"	Methaemoglobin. Traces of Oxyhaemoglobin.
Haemoglobin Compound	Liquid	"	Burnt Malt	Sweet, alcoholic, malty	Readily miscible in water	Acid	.015		Mixt. of Methaemoglobin and Oxybaemoglobin.